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BEHAVIOURAL SENSITIZATION AND TOLERANCE TO COCAINE: AN INVESTIGATION OF DOPAMINE AND SEROTONIN MECHANISMS

by

© LYNN YVONNE BURGER

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
PHARMACEUTICAL SCIENCES
FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES
AND FACULTY OF MEDICINE (PSYCHIATRY)

EDMONTON, ALBERTA

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Atomic	0748
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Elementary Particles and High Energy	0798
Fluid and Plasma	0759
Molecular	0609
Nuclear	0610
Optics	0752
Radiation	0756
Solid State	0611
Statistics	0463

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Automotive	0540
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System Science	0790
Geotechnology	0428
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To Mum, Dad and Julie,

in this our 20th year as Canadians

and

To Allen,

my best friend

ABSTRACT

Chronic administration of the psychomotor stimulant cocaine results in the development of behavioural tolerance if given continuously or sensitization with intermittent daily injections. This dissociation of behavioural effects has been linked to the effect cocaine has on monoamine systems, particularly dopamine (DA). Studies were undertaken to investigate the role of DA and serotonin (5-HT) in mediating behavioural sensitization and tolerance to cocaine using the receptor alkylating agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). EEDQ denatures DA D1 and D2 and 5-HT$_2$ types that are not protected by endogenous or exogenous ligands and therefore enables measurement of occupied receptors *in vivo*. Chronic cocaine given continuously was found to increase the occupation of D1, but not D2, receptors in rat striatum, depending on the circadian cycle. This effect was reversed by co-administration of a D1 antagonist suggesting a phasic role for D1 receptors in response to continuous cocaine. A detailed analysis of behaviour revealed tolerance to the locomotor- and stereotypy-inducing properties of cocaine, regardless of the day/night cycle. Direct comparison of the effects of cocaine treatment regimen on behaviour was also examined in animals receiving either continuous or intermittent treatment for 2 weeks, followed by either a 0 or 10 day withdrawal period from cocaine. Intermittent cocaine produced behavioural sensitization that was accompanied by increased occupation of D1 and D2 receptors in the striatum and nucleus accumbens (NAS). This effect was not seen in animals exhibiting tolerance to continuous cocaine treatment, or those pre-treated with nimodipine, an L-type Ca^{2+} channel blocker known to block cocaine's ability to facilitate the impulse-dependent release of DA. After a 10 day withdrawal period, no effect of pretreatment regimen on cocaine-induced DA receptor occupation was found, suggesting dispositional differences between the two treatment regimens. Similar findings were reported for 5-HT$_2$ receptor occupation in the frontal cortex. It is concluded that cocaine given intermittently enhances the impulse-dependent release of DA and 5-HT, since nimodipine successfully blocks this effect in both systems. However, increased DA release in the striatum and increased 5-HT release in the frontal cortex do not mediate behavioural sensitization to cocaine, as measured by protection from EEDQ denaturation.

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TABLE OF CONTENTS

CHAPTER 1
GENERAL INTRODUCTION 1

1.1 PHARMACOLOGY OF THE PSYCHOMOTOR STIMULANT COCAINE 1
1.2 BEHAVIORAL SENSITIZATION AND TOLERANCE TO PSYCHOMOTOR STIMULANTS 4
  1.2.1 Tolerance 5
  1.2.2 Sensitization 7
1.3 BEHAVIOURAL SENSITIZATION AS AN ANIMAL MODEL OF STIMULANT-INDUCED PSYCHOSIS . . . 10
1.4 MECHANISMS OF STIMULANT SENSITIZATION 11
  1.4.1 Drug dispositional effects 11
  1.4.2 Dopaminergic substrates 12
    1.4.2.1 Postsynaptic changes 12
    1.4.2.2 Presynaptic changes 13
  1.4.3 Serotonergic transmission 15
1.5 RECEPTOR ALKYLATION AS A TOOL IN THE INVESTIGATION OF NEURAL SUBSTRATES OF COCAINE-INDUCED SENSITIZATION 16
1.6 THESIS OBJECTIVES 18
1.7 REFERENCES 20

CHAPTER 2
EEDQ AS A TOOL FOR ASSESSING RECEPTOR FUNCTION: A DOSE-RESPONSE ANALYSIS WITH COCAINE 39

2.1 INTRODUCTION 39
2.2 METHODS 40
  2.2.1 Animals 40
  2.2.2 Drug Administration 41
  2.2.3 Tissue Collection 41
  2.2.4 DA Receptor Binding 42
  2.2.5 Protein Determination 43
  2.2.6 HPLC Analysis 43
  2.2.7 Statistics 44
2.3 RESULTS 45
  2.3.1 DA Receptor Binding 45
  2.3.2 DA Turnover 48
2.4 DISCUSSION 48
2.5 REFERENCES 53

CHAPTER 3
DAY/NIGHT DIFFERENCES IN D1 BUT NOT D2 DA RECEPTOR PROTECTION FROM EEDQ DENATURATION IN RATS TREATED WITH CONTINUOUS COCAINE 56

3.1 INTRODUCTION 56
3.2 MATERIALS AND METHODS 59
  3.2.1 Animals 59
  3.2.2 Drug Administration Procedure 59
  3.2.3 DA Receptor Binding 60
  3.2.4 Statistics 60
3.3 RESULTS 61
  3.3.1 D1 Receptor Binding 61
  3.3.2 D2 Receptor Binding 67
3.4 DISCUSSION 70
3.5 REFERENCES 79

CHAPTER 4
THE EFFECTS OF CONTINUOUS COCAINE ADMINISTRATION ON LOCOMOTION AND THE STRUCTURE OF BEHAVIOUR 86

4.1 INTRODUCTION 86
4.2 METHODS 89
  4.2.1 Animals, apparatus and drug 89
  4.2.2 Surgical Procedures 90
  4.2.3 Behavioural analysis 90
  4.2.4 Statistics 91
4.3 RESULTS 94
  4.3.1 Locomotor Activity 94
  4.3.2 Behavioral analysis: Frequencies and Durations 97
  4.3.3 Behavioural Transition Analysis 103
4.4 DISCUSSION 109
4.5 REFERENCES 117

CHAPTER 5
BEHAVIOURAL SENSITIZATION AND TOLERANCE TO COCAINE AND THE OCCUPATION OF DA RECEPTORS BY DA 122

5.1 INTRODUCTION 122
5.2 METHODS 124
  5.2.1 Animals 124

5.2.2 Drug Administration Procedure 125
5.2.3 DA Receptor Binding 126
5.2.4 Statisitics 126
5.3 RESULTS 127
5.3.1 Pre-Withdrawal 127
5.3.2 Post-Withdrawal 135
5.4 DISCUSSION 140
5.5 REFERENCES 146

CHAPTER 6
CHRONIC COCAINE ADMINISTRATION DOES NOT AFFECT 5HT$_2$ RECEPTOR OCCUPATION IN RAT FRONTAL CORTEX 152

6.1 INTRODUCTION 152
6.2 METHODS 155
6.2.1 Animals 155
6.2.2 Drug Adminstration Procedure 155
6.2.3 5-HT$_2$ Receptor Binding 156
6.2.4 Statistics 157
6.3 RESULTS 157
6.4 DISCUSSION 161
6.5 REFERENCES 168

CHAPTER 7
GENERAL DISCUSSION 174

7.1 CONCLUSIONS 181
7.2 FUTURE DIRECTIONS 182
7.3 REFERENCES 184

APPENDIX A. GENERAL METHODS 188

A.1 CHEMICALS USED 189
A.2 INSTRUMENTATION AND APPARATUS 191
A.2.1 Filtration. 191
A.2.2 Centrifuges 191
A.2.3 Tissue Homogenizer 191
A.2.4 Weighing Balances 191
A.2.5. Glass Cleaning 191

APPENDIX B. BEHAVIOUR DURATIONS (TABLE 1) AND FREQUENCIES (TABLE 2) 193

LIST OF TABLES

Table 2-1. K_d values for D1 and D2 receptors in striatum after EEDQ 49

Table 3-1. Average K_d values for D1 and D2 receptors in striatum following chronic cocaine and/or SCH 23390 and/or raclopride 68

Table 4-1. Operational definitions of behaviours assessed in BEBOP 92

Table 4-2. Behavioural transitions between behaviours in the EXPLORE category after chronic cocaine treatment 104

Table 4-3. Behavioural transitions between behaviours in the QUIET category after chronic cocaine treatment 108

Table 6-1. 5-HT_2 receptor K_d values after chronic cocaine treatment given continuously or intermittently, with or without nimodipine 159

Table 6-2. 5-HT_2 receptor K_d values after a 10 withdrawal period from chronic cocaine given continuously or intermittently with or without nimodipine 162

LIST OF FIGURES

Fig. 2-1. Striatal D1 DA receptor density after EEDQ alone or in combination with cocaine 46

Fig. 2-2. Striatal D2 DA receptor density after EEDQ alone or in combination with cocaine 47

Fig. 2-3. DA turnover in olfactory tubercle after EEDQ alone or in combination with cocaine 50

Fig. 3-1. DA D1 receptor occupation in the striatum after continuous cocaine treatment 62

Fig. 3-2. DA D1 receptor occupation following administration of chronic cocaine and the D1 antagonist SCH23390 63

Fig. 3-3. DA D1 receptor occupation following administration of chronic cocaine and the D2 antagonist raclopride 65

Fig. 3-4. DA D1 receptor occupation following administration of chronic cocaine, SCH23390 and raclopride 66

Fig. 3-5. D2 receptor density in rat striatum as a function of raclopride treatment 69

Fig. 4-1. Daytime cocaine-induced locomotion over 14 days 95

Fig. 4-2. Nocturnal cocaine-induced locomotion over 14 nights 96

Fig. 4-3. EXPLORE frequencies and durations on first and last days of cocaine treatment 99

Fig. 4-4. INTERMEDIATE frequencies and durations on first and last days of cocaine treatment 100

Fig. 4-5. QUIET frequencies and durations on first and last days of cocaine treatment 102

Fig. 5-1. Locomotor activity over 14 consecutive days after in animals treated continuously or intermittently with cocaine and/or nimodipine 128

Fig. 5-2 Locomotor activity prior to EEDQ administration in animals treated continuously or intermittently with cocaine and/or nimodipine 129

Fig. 5-3. DA D1 receptor density in the striatum after chronic cocaine given continuously or intermittently with or without nimodipine co-treatment 131

Fig. 5-4. DA D2 receptor density in the striatum after chronic cocaine given continuously or intermittently with or without nimodipine co-treatment 132

Fig. 5-5. DA D1 receptor density in the NAS after chronic cocaine given continuously or intermittently with or without nimodipine 133

Fig. 5-6. DA D2 receptor density in the NAS after chronic cocaine given continuously or intermittently with or withour nimodipine 134

Fig. 5-7. Locomotor activity in animals receiving cocaine continuously or intermittently with or without nimodipine over 10 consecutive days . . 136

Fig. 5-8. Locomotion after a cocaine challenge on Day 10 of cocaine withdrawal after continuous or intermittent treatment with or without nimodipine 137

Fig. 5-9. DA D1 receptor density in rat striatum after withdrawal from continuously or intermittently administered cocaine with or without nimodipine . . . 138

Fig. 5-10. DA D2 receptor density in rat striatum after withdrawal from continuously or intermittently administered cocaine with or without nimodipine . . . 139

Fig. 6-1. 5-HT_2 receptor density in rat frontal cortex after chronic cocaine administered continuously or intermittently, with or without nimodipine 158

Fig. 6-2. 5-HT_2 receptor density in rat frontal cortex after 10 days withdrawal from chronically adminstered cocaine and nimodipine 160

TABLE OF ABBREVIATIONS

ANOVA	analysis of variance
Bmax	maximum density of binding sites (fmol/mg protein)
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
DA	dopamine
DPM	disintegrations per minute
DRN	dorsal raphe nucleus
EEDQ	N-ethoxycarbonyl-2-ethoxy-1,2-dihyrodroquinoline
fmol	femtomole (10^{-15} moles)
g	gram
GABA	γ-aminobutryic acid
h	hour
HPLC	high pressure liquid chromatography
5-HT	5-hydroxytryptamine (serotonin)
HVA	homovanillic acid
i.p.	intraperitoneally
K_d	dissociation constant (in nM)
kg	kilogram
l	litre
LC	locus coeruleus
M	molar
mg	milligram
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
NA	noradrenaline
NAS	nucleus accumbens
nM	nanomolar
6-OHDA	6-hydroxydopamine
PFC	prefrontal cortex
PHNO	(+) 4-propyl-9-hyroxynaphthoxazine
rpm	revolutions per minute
sec	second
SEM	standard error of the mean
SN	substantia nigra
Tris	Tris buffer
vols	volumes
VTA	ventral tegmental area
µl	microlitre
µM	micromolar
°C	degree Celsius

CHAPTER 1. GENERAL INTRODUCTION

1.1. PHARMACOLOGY OF THE PSYCHOMOTOR STIMULANT COCAINE

Classification of cocaine as a psychomotor stimulant is based on its ability to elicit increased motoric activity. This effect is seen at low doses where, like other stimulants, cocaine induces increased locomotion, rearing, grooming and overall exploration (Scheel-Kruger et al., 1977; Snoddy and Tessel, 1985). With increasing doses, locomotor activity decreases as specific behavioural patterns become stereotyped i.e. repetitive and "purposeless" in nature. In rats, stereotypy includes repetitive head movements, sniffing, gnawing, licking and biting (Scheel-Kruger, 1971; Scheel-Kruger et al., 1977). The locomotor stimulation effects of cocaine have been proposed to originate from activation of a dopamine (DA) pathway anatomically distinct from the pathway that mediates stereotypy and, as a result, these two behavioural consequences of cocaine are in direct competition (Lyon and Robbins, 1975; Joyce and Iversen, 1984). In comparison to amphetamine, cocaine-induced stereotypy has been characterized as less perseverative (Scheel-Kruger et al., 1977).

As a central nervous system (CNS) stimulant, cocaine also has reinforcing properties as it is readily self-administered by both human and nonhuman animals (Fischman, 1984; Pickens and Thompson, 1968). The rewarding aspects of cocaine use have led to its widespread abuse in today's society and have subsequently limited its medical use as a local anesthetic and vasoconstrictor (Johanson and Fischman, 1989). The local anesthetic potency of cocaine is equal to that of lidocaine (Goodman and Gilman,

1965) and provides cocaine with pharmacological properties that distinguish it from other psychomotor stimulants. While psychomotor stimulation is seen at low cocaine doses (5-10 mg/kg), higher doses (50-65 mg/kg) are required to produce the local anesthetic properties (Post et al., 1992). This local anesthetic effect is responsible for the convulsive effects of repeated cocaine exposure, or after a single high dose (Weiss et al., 1989).

The neurochemical basis of cocaine's motor activating effects is thought to be attributable to its ability to block the uptake of monoamines (Ross and Renyi, 1967, 1969; Friedman et al., 1975; Langer and Enero, 1974), particularly DA (Heikkila et al., 1975; Ross and Renyi, 1967), at synaptic membranes. This action is accomplished by binding to the transporter site at these synapses (Schoemaker et al., 1985; Kennedy and Hanbauer, 1983; Reith et al., 1983), although evidence for [^{3}H]cocaine binding to noradrenaline (NA) uptake sites has been difficult to provide, possibly due to the rapid dissociation of [^{3}H]cocaine from NA sites at the temperature used in the filtration assay (Reith, 1992). Cocaine subsequently increases monoamine concentrations in the extracellular space to potentiate neurotransmission in various brain regions (Pradhan et al., 1978; Hurd and Ungerstedt, 1989; Kalivas and Duffy, 1990; Galloway, 1990). The acute and transient enhancement of DA neurotransmission is an effect shared by other psychomotor stimulants, including amphetamine, which causes DA release (Heikkila et al., 1975; Baumann and Maitre, 1976). This increase in extracellular DA concentrations has been shown to correlate with the locomotor and stereotypy-inducing properties of the psychomotor stimulants (Kuczenski and Segal, 1989; Carboni et al., 1989; Kalivas and Duffy, 1990; Kiyatkin, 1993). Further evidence for a dopaminergic role in mediating the behavioural effects of stimulants stems from studies showing that 6-hydroxydopamine (6-OHDA)-induced lesions of the mesolimbic DA pathway abolish stimulant-induced locomotion (Kelly and Iversen, 1975) as does administration of DA antagonists (Ljundberg and Ungerstedt, 1985). The reinforcing properties of cocaine have also been shown to be associated witth enhanced synaptic DA in mesocorticolimbic DA neuronal pathways (Wise, 1984). Blockade of cocaine self-adminstration with neurotoxic lesions in the nucleus accumbens (NAS) (Roberts et al., 1977, 1980) or by direct injections of DA antagonists into this DA region (Philips et al., 1983) has also been demonstrated.

The neuropharmacologic effects of cocaine, as a consequence of increased monoamine transmitter levels, include activation of negative feedback mechanisms which suppress unit activity in cell body regions. This inhibition has been demonstrated in spontaneously firing single noradrenergic locus coeruleus (LC) (Pitts and Marwah, 1986, 1987), serotonergic dorsal raphe´ nucleus (DRN) (Lakoski and Cunningham, 1988; Pitts and Marwah, 1986, 1987), dopaminergic substantia nigra (SN) (Pitts and Marwah, 1986, 1987) and ventral tegmental area (VTA) (Pitts and Marwah, 1986, 1987; White et al., 1987; Einhorn et al., 1988; White et al., 1992) neurons. This compensatory mechanism is also accompanied by inhibited monoamine synthesis following cocaine administration (Galloway, 1990; Taylor and Ho, 1977). For DA at least, synthesis inhibition appears to be mediated by cocaine-induced D2 autoreceptor stimulation since this effect is blocked by the D2 antagonist sulpiride (Galloway, 1990). Suppression of DA cell firing stems from activation of cell body autoreceptors (Argiolas et al., 1982; Savasta et al., 1986) as well postsynaptic stimulation of the long-loop γ-aminobutyric acid (GABA)-ergic projection from the striatum to the SN (Carlsson and Lindquist, 1963; Gerfen and Young, 1988; Nagy and Fibiger, 1980). The mechanism by which cocaine inhibits serotonin (5-HT) synthesis remains to be determined. Taken together, these actions at monoaminergic nerve terminals result in cocaine-induced increases in neurotransmitter levels followed by subsequent compensatory mechanisms to decrease cell firing and synthesis. This sequence of events serves to terminate cocaine's acute effects.

Although DA is believed to be the most important system in mediating the locomotor and stereotypy-inducing effects of cocaine, the role of NA and 5-HT should not be overlooked. Noradrenergic lesions, for example, have been shown to enhance amphetamine-induced stereotypy (Segal and Schuckit, 1983). With respect to serotonergic mediation of stimulant-induced behaviours, potentiation of cocaine's locomotor enhancing effects has been observed after inhibition of 5-HT synthesis or blockade of 5-HT receptors (Scheel-Kruger et al., 1977). Interactions between 5-HT and DA systems could also be involved in the behavioural effects of cocaine (Galloway, 1992). Research on the behavioural pharmacology of cocaine is only now beginning to define the precise mechanisms of action of cocaine administration. Further examination of the chronic effects of these drugs is an area of research with particular clinical importance, given that human patterns of use generally involve repeated administration.

1.2. BEHAVIOURAL TOLERANCE AND SENSITIZATION TO PSYCHOMOTOR STIMULANTS

The concepts of tolerance and sensitization in the study of drug effects refer to shifts in the dose-response curve with repeated administration. Sensitization is represented as a shift to the left, with tolerance seen as a shift to the right. These phenomena are not mutually exclusive since tolerance may develop to one effect of the drug as sensitization emerges to another. Repeated administration of psychomotor stimulants, for example, has been associated with the development of tolerance to the anorectic and hypothermic effects with sensitization to the stereotypy, locomotor, convulsive and dyskinetic properties of these drugs (Post and Contel, 1983). These changes in the effectiveness of a drug over time may be due to a number of different processes, including drug dispositional changes (Le and Khanna, 1989), changes at the target site (Klein et al., 1989), inter-neuronal changes (see Goudie and Emmett-Oglesby, 1989 for review) and classical conditioning of drug effects (see Martin-Iverson et al., 1993 for review). In the end, these mechanisms involve changes that either adjust the system to a pre-drug state (tolerance) or facilitate the drug effect (sensitization) with further administration.

1.2.1. Tolerance

Tolerance is formally defined as a shift to the right in the dose-response function. Behavioural tolerance to the stimulant properties of cocaine emerges after chronic administration in which the drug is delivered continuously (King et al., 1992; Reith et al., 1987). Essentially, initial increases in locomotor activity on the first days of drug exposure gradually decline to levels indistinguishable from vehicle controls. Upon subsequent drug challenge, the level of locomotor activity elicited by the same dose on Day 1 of delivery is now significantly diminished. Behavioural tolerance, as it is interpreted in this thesis, therefore refers to tolerance to the behavioural effects of stimulants and not to behavioural mechanisms of tolerance. These behavioural effects have also been reported with continuous infusion of amphetamine and the D2 agonist (+)-4-propyl-9-hydroxynaphthoxazine (PHNO) (Martin-Iverson, 1988a,b) during the day.

The neurobiology of behavioural tolerance to cocaine has been linked to changes in the DA system. Animals with a history of continuous administration of cocaine have shown decreased DA efflux measured *in vitro* after a cocaine challenge (King et al., 1993). This finding raises the possibility that tolerance to psychomotor stimulant effects on motor activity may be due to a decrease in DA release. Classically, tolerance to drugs is explained as being due to decreased receptor density (subsensitivity), thereby resulting in diminished behavioural response. For the psychomotor stimulant drugs, however, this mechanism may not be important in the development of behavioural tolerance. Tolerance to PHNO, for example, occurs only during the day while sensitization is observed at night. This switch in behavioural activity occurs within one hour and is therefore too rapid to be accounted for by changes in receptor density (Martin-Iverson et al., 1988a). Furthermore, daytime tolerance can be reversed by stress which, in turn, can be either blocked by a D1 antagonist or mimicked by a D1 agonist (Martin-Iverson et al., 1988b). These data indicate that tolerance results from a decrease in DA release at D1 receptor sites.

Behavioural tolerance to continuous psychomotor stimulants has also been explained as a neurotoxic effect on DA systems (Robinson and Becker, 1986). For PHNO, however, continuous infusion actually increases DA content in the brain (Martin-Iverson, 1991b). In addition, cocaine does not lead to striatal neurotoxicity, even at extremely large doses (Kleven et al., 1988; Ryan et al., 1988). Most damaging to a DA depletion hypothesis of stimulant tolerance is the lack of effect on *in vivo* extracellular DA levels after long term DA depletions induced by neurotoxic doses of methamphetamine (Robinson et al., 1990). If tolerance to psychomotor stimulants is a function of a general phenomenon common to all of these drugs, it appears to be a result of decreased DA release, possibly related to decreases in impulse activity.

1.2.2. Sensitization

Repeated administration of cocaine and other psychomotor stimulants can also augment the behavioural effects seen with acute doses. Increased levels of locomotor activity (Shuster et al., 1977) as well as greater intensity of stereotypies (Kilbey and Ellinwood, 1977a; Post and Rose, 1976; Stripling and Ellinwood, 1977) have been reported after chronic administration. This behavioural sensitization occurs in a number of animal species, including rats (Post and Rose, 1976), cats (Ellinwood et al., 1977) and mice (Reith, 1986). Behavioural sensitization to cocaine is a stable and enduring phenomenon that can be elicited by a subsequent challenge dose weeks or even months after cessation of treatment.

The emergence of behavioural sensitization depends on several factors associated with the regimen of drug adminstration. Dose and the number of days of cocaine administration are both important determinants of sensitization. As summarized by Post et al. (1992), a 10 mg/kg dose of cocaine given for 10 days induces behavioural sensitization that is present months later. A single injection at this dose, followed later with a challenge dose, does not induce any augmentation in behaviour but a 40 mg/kg treatment results in locomotor sensitization that lasts for several days. The effects of cocaine therefore appear to be more persistent at either higher doses or longer durations of treatment.

The time interval between drug administrations is also a crucial variable that determines the direction of behavioural adaptation to repeated cocaine. Intermittent delivery *via* daily intraperitoneal or subcutaneous injections is associated with augmented levels of activity and stereotypy over time, but continuous administration *via* subcutaneously implanted pellets or pumps generally induces behavioural tolerance (Reith et al., 1987; Post et al., 1981; King et al., 1992). Continuous chronic drug delivery to maintain relatively constant plasma concentrations is therefore ineffective in producing heightened activity upon subsequent cocaine challenge, a finding that also holds true for amphetamine (Nelson and Ellison, 1978; Neilson, 1981; Martin-Iverson, 1991a), methylphenidate (Wood et al., 1977) and PHNO (Martin-Iverson et al., 1987, 1988a,b, 1991a). Time intervals between injections has also been shown to influence the strength of behavioural sensitization to amphetamine (Post, 1980; Antelman and Chiodo, 1981), but the importance of this factor has not been elucidated for cocaine as of yet.

For sensitization to cocaine to occur, some research has suggested that classical conditioning and environmental context are important. Post et al. (1981) and Reimer and Martin-Iverson (1994) demonstrated that enhanced responsivity to cocaine is observed only in animals that received cocaine in the context of the test cage as opposed to those previously receiving cocaine in another environment. Context specificity has been shown for amphetamine (Drew and Glick, 1988; Tilson and Rech, 1973; Stewart and Vezina, 1991), cocaine (Barr et al., 1983; Beninger and Herz, 1986; Hinson and Poulos, 1981;

Post et al., 1981) and apomorphine (Mattingly and Gotsic, 1989). This sensitization has also been shown to be subject to extinction procedures since repeated saline injections in the previously associated cocaine environment eliminates behavioural sensitization (Hinson and Poulos, 1981). The significance of the environmental context is also highlighted by the lack of sensitization in paradigms with continuous infusion of drug where contextual cues uniquely associated with drug administration are not present. It should be noted, however, that when behaviour is examined over the entire circadian cycle, sensitization occurs at night with continuous infusion of amphetamine or PHNO (Martin-Iverson, 1991a; Martin-Iverson et al., 1988a,b). Therefore sensitization can occur in the absence of conditioning to specific cues associated with drug administration. Context-independent sensitization has also been demonstrated for cocaine by Post and Weiss (1988), who suggest that effects of higher doses are less dependent on environmental context.

Individual differences, including age, sex and genetic makeup have also been found to influence behavioural sensitization. More intense cocaine-induced behaviours have been documented in old than in young rats (Kilbey and Ellinwood, 1977b). This finding was supported by Post and Contel (1983) who report greater increases in horizontal and, in particular, vertical activity in older rats. With respect to sex differences in cocaine response, females are more sensitive to acute doses and require half the dose given to males to induce behavioural sensitization (Post and Contel, 1983). This same finding holds true for amphetamine-induced sensitization (Camp and Robinson, 1985; Robinson et al., 1982) and is extended to include heightened sensitization in castrated compared to normal males, suggesting that gonadal hormones may influence sensitization. Finally, Shuster et al. (1977) found differing degrees of behavioural sensitization to cocaine in different genetic strains of mice. Subtle differences in the underlying neural and biochemical mechanisms may therefore influence the adaptive response to chronic stimulant administration.

1.3. BEHAVIOURAL SENSITIZATION AS AN ANIMAL MODEL OF STIMULANT-INDUCED PSYCHOSIS

Much of the interest in the effects of chronic stimulant administration is based on the finding that repeated treatment in humans can induce psychotic episodes viritually indistinguishable from paranoid schizophrenia (Connell, 1958; Angrist,1983; Segal and Schuckit, 1983). This psychosis has been reported with cocaine abuse (Cox et al., 1983). Adminstration of stimulants to schizophrenics leads to a worsening of their psychotic symptoms and can induce a psychotic episode in patients with a history of stimulant abuse (Angrist, 1983; Segal and Schuckit, 1983). The use of nonhuman animals as models of stimulant-induced psychosis provides researchers with a valuable tool with which to investigate schizophrenic and drug-induced psychoses. Direct comparisons of motor sensitization in animals and psychopathology in humans do not, on the surface, reveal obvious similarities due to species differences in behaviour. Behavioural correlates can, however, be achieved in animal models as disruptions in normal behavioural patterns, and "hallucinatory"-like behaviours emerge with chronic stimulant administration (Ellison, 1991). These behaviours include formication or excessive forelimb grooming as well as abortive grooming and limb flicks, which are observed in animals following administration of hallucinogenic compounds like lysergic acid (LSD). The search for neural substrates of stimulant-induced behaviour is probably the most important rationale for using animal models in this area of research. An examination of the mechanisms of cocaine-induced sensitization has clinical implications in that it may provide information about the neuropathology associated with stimulant-induced psychosis and schizophrenia.

1.4. MECHANISMS OF STIMULANT-INDUCED SENSITIZATION

1.4.1. Drug dispositional effects

The possibility that dispositional effects of stimulant administration may influence sensitization has been explored. Research has generally concluded that changes in the pharmacokinetics of amphetamine or the presence of active metabolites cannot account for the augmented behavioural response with repeated administration (Robinson and Becker, 1986; Kuczenski et al., 1982). For cocaine, however, repeated administration has been shown to change the pharmacokinetics of subsequent drug administration in which sensitized animals have higher cocaine levels in the brain compared to naive controls (Reith et al., 1987). However, no differences between cocaine-sensitized and cocaine-tolerant animals was found with a cocaine challenge after a drug withdrawal period thereby refuting dispositional theories of sensitization. Considering that sensitization is a long-lasting phenomenon (Kalivas et al., 1988) and can be instilled after only one injection of cocaine (Lin-Chu et al., 1985), a very short acting compound (Johanson and Fischman, 1989), it is unlikely that kinetic changes are the underlying mechanism. The finding of cross-sensitization among compounds with different pharmacological profiles (e.g. cocaine to apomorphine, Kilbey and Ellinwood, 1977a) provides further evidence for a nondispositional substrate of sensitization.

1.4.2. Dopaminergic substrates

The search for biochemical correlates of stimulant-induced sensitization has resulted in a complex picture of neurotransmitter effects. Most attempts to identify a neural substrate of behavioural sensitization have focused on the DA system, with investigation primarily of the nigrostriatal and mesoaccumbens DA projections. The results of analyses into both pre- and postsynaptic changes in these major pathways have often produced equivocal findings, with different results depending on the type of psychomotor stimulant administered and the duration of withdrawal after repeated administration (Kalivas et al., 1993). However, certain neurochemical changes in response to chronic stimulant administration are reliably elucidated in these DA projections and, with more research, may provide definitive clues to a neural mechanism of sensitization.

1.4.2.1. Postsynaptic changes: The effect of chronic stimulant administration on DA postsynaptic receptor density was first shown to result in supersensitivity based on striatal receptor binding and hypersensitivity to apomorphine after repeated amphetamine administration (Klawans and Margolin, 1975). However, much of the more recent work with amphetamine has failed to support this notion and some have even proposed receptor down-regulation with amphetamine-induced sensitization (Robinson and Becker, 1986). This inconsistency in receptor effects was also found in the mesoaccumbens projection. More recently, the effects of chronic cocaine on postsynaptic receptors has shown decreases in D2 receptor densities in the striatum and increases in the nucleus accumbens (Goeders, 1990; Kleven et al., 1990; Peris et al., 1990). Electrophysiological evidence for changes in receptor function with cocaine have revealed increased sensitivity of both D1 and D2 receptors in the nucleus accumbens (Henry et al., 1989). Further examination revealed that the increase in D1 sensitivity was not mediated by changes in receptor density (Kleven et al., 1990) but did persist in the nucleus accumbens for at least 1 month after cessation of treatment (Henry and White, 1991). Kleven et al. (1990), on the other hand, report long-lasting decreases in D1 receptor density in the striatum after 15 days of cocaine. The search for a postsynaptic mechanism of sensitization has therefore produced mostly transient effects, with the more enduring changes associated with D1 receptor function.

1.4.2.2. Presynaptic changes: Given that sensitization is induced by direct injections of stimulants into the cell body, but not the terminal, regions of the DA system (Kalivas and Weber, 1988; Vezina and Stewart, 1990; Vezina, 1993; Dougherty and Ellinwood, 1981; Hitzemann et al., 1980) changes in presynaptic functions have been implicated in the effects of chronic administration of such drugs. Measurements of the extracellular concentration of DA in the striatum and nucleus accumbens reveal either no significant changes (Kuczenski and Leith, 1981) or decreased levels (Nishikawa et al., 1983) in amphetamine-sensitized animals subjected to drug challenge. Parsons et al. (1991) report decreased basal levels of DA after withdrawal from chronic cocaine while Pettit et al. (1990) found heightened DA levels in the NAS prior to abstinence . Other laboratories failed to find any significant changes in basal extracellular DA levels regardless of withdrawal times (Robinson et al., 1988; Segal and Kuczenski, 1992 a,b; Kalivas and Duffy, 1993). The data on DA concentrations following psychostimulant administration are therefore inconsistent. The same conclusion holds for studies on DA synthesis. Most often, amphetamine was found to not influence the synthesis of DA (Nishikawa et al., 1983), but others report increased (Kuczenski and Leith, 1981) or decreased (Taylor and Ho, 1977; Besson et al., 1973) synthesis in the striatum. Decreased DA synthesis has been reported with chronic cocaine (Brock et al., 1990). Investigations of D2 autoreceptor function have also yielded equivocal results, with reports of decreases (Yi and Johnson, 1990), increases (Dwoskin et al., 1988), or no change (Fitzgerald and Reid, 1991) following repeated cocaine. The most enduring and consistent change in presynaptic DA function has been the finding of enhanced DA release in terminal regions in response to psychomotor stimulant challenge. This is confirmed by *in vitro* studies of amphetamine- or electrically-stimulated DA release in amphetamine- (Robinson and Becker, 1982; Castaneda et al., 1988) and cocaine- (Peris and Zahniser, 1987; Kalivas and Duffy, 1988) sensitized animals. These data concur with more sophisticated *in vivo* experiments of stimulant-induced sensitization (Robinson et al., 1988; Akimoto et al., 1989, 1990; Pettit et al., 1990; Kalivas and Duffy, 1990, 1993). There are some studies, however, that fail to verify the association between sensitization and increased DA release (Hurd et al., 1989; Segal and Kuczenski, 1992a,b; Weiss et al., 1992). A closer look at the time course of these experiments suggests that the withdrawal period of 48 hours was not sufficient to detect any change in DA levels. The augmentation in stimulated DA release has been shown to be related to the withdrawal period such that greater effects are found 1 week or more compared to 24 hours after cessation of stimulant treatment (Kalivas and Duffy, 1989; Robinson, 1991; Hitzemann et al., 1977). This time-dependent feature of augmented DA release was studied further and revealed that although animals subjected to chronic cocaine exhibited behavioural sensitization at both 24 hours and 2-3 weeks of withdrawal, elevated extracellular DA was detected only at the latter time sample. This discrepancy may be due to enhanced DA uptake in early versus late withdrawal from cocaine (Kalivas and Duffy, 1993; Parsons et al., 1991), a process which could transiently mask the enhanced DA release and prevent detection with *in vivo* microdialysis (Parsons and Justice, 1993a). Conclusive evidence for a relationship between DA release and sensitization is therefore difficult to obtain using the *in vivo* microdialysis technique. Finally, a close look at the role of DA neurons in the VTA mediating behavioural sensitization to cocaine has shown heightened basal firing rates after chronic treatment (Henry et al., 1989), a finding that may be related to increased DA release from DA terminals. D1 receptor effects are once again implicated in this presynaptic effect since direct injections of the D1 antagonist SCH23390 into the VTA and SN attenuate the development of sensitization to amphetamine injections (Stewart and Vezina, 1989).

1.4.3. Serotonergic transmission

It has been proposed that psychomotor stimulant-induced paranoid psychosis may be related to alterations in serotonergic function (Bleich et al., 1988). Cocaine, as previously described, is a potent inhibitor of 5-HT uptake and subsequently leads to a dampening of unit activity in the DRN. It has been argued that this altered electrophysiological sensitivity of 5-HT cells to 5-HT$_{1A}$ autoreceptor stimulation represents a change in receptor transduction mechanisms in cocaine-sensitized animals (Cunningham et al., 1992 a, b). There is also evidence of increased ^{3}[H]imipramine binding suggesting an increase in 5-HT uptake sites in the cortex after chronic cocaine administration (Cunningham et al., 1992a,b). A recent microdialyis study also points to enhanced extracellular 5-HT levels in the nucleus accumbens, VTA and DRN in cocaine-sensitized animals upon subsequent cocaine challenge (Parsons and Justice, 1993b). These data point to a possible role for 5-HT release in the expression of behavioural sensitization.

1.5. RECEPTOR ALKYLATION AS A TOOL IN THE INVESTIGATION OF NEURAL SUBSTRATES OF COCAINE-INDUCED SENSTIZATION

The search for a mechanism of behavioural sensitization to psychomotor stimulants has involved a variety of neurochemical techniques but, to date, no research exists using the method of receptor alkylation to examine the occupation of receptor sites after chronic adminstration of cocaine. Receptor occupation (not overall density) allows for an assessment of the amount of neurotransmitter available for binding in the extracellular space. In other words, this method gives an indirect measure of neurotransmitter release *in vivo*.

Receptor alkylation is achieved with administration of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), a compound that reacts with carboxyl groups at the receptor site and, through a highly reactive intermediate, creates a covalent bond to prevent further binding and occupation of any exogenous or endogenous ligand. More specfically, EEDQ inactivates carboxyl groups through the formation of a mixed carbonic anhydride intermediate which, after attack by a nucleophillic group at the receptor site, results in the formation of a cross-linkage between the sterically accessible nucleophile and the original carboxyl function (Belleau et al., 1968, 1969; Belleau and Malek, 1968). This action is believed to take place at the external surface of cell membranes since EEDQ does not alter intracellular mediators that stimulate adenylate cyclase at the D1 receptor site (Meller et al., 1986). In addition, EEDQ has been found to have greater potency when used with *in vitro* versus *in vivo* protocols, possibly due to its ability to gain intraneuronal access only in tissue homogenates (Meller et al., 1986). Peripheral administration of EEDQ has been associated with changes in receptor Bmax but not K_d, supporting a non-equilibrium mechanism of receptor antagonism (Hamblin and Creese, 1983). Essentially, EEDQ acts to denature receptors that are vacant or unprotected with bound ligands in a noncompetitive fashion. At equilibrium, the presence of a ligand at or near the receptor site will likely prevent EEDQ from inactivating that site due to the fact that the rates of association are equal to the rates of dissociation between the receptor and its ligand. Receptor protection from EEDQ is therefore offered in the presence of some endogenous or exogenous ligand, preventing the total denaturation of selective receptors. Since EEDQ inactivation is irreversible, *in vitro* binding assays result in the labelling of only those receptors previously protected and therefore give an estimate as to the relative occupation of receptors at the time of administration.

EEDQ denatures a number of different monoamine receptors, including α-adrenergic, dopaminergic D1 and D2, 5-HT_2, and 5-HT_1 types (Meller et al., 1986). This nonselectivity makes EEDQ a valuable tool for assessing different receptor changes in the same animal model. EEDQ administration also offers the advantage of being much less invasive to the functioning brain than other methods that examine extracellular events. *In vivo* microdialysis, for example, involves major surgery with resultant brain damage from the placement of the probe, two limiting factors that create problems in interpreting results. This particular technique is also limited by its regional and neurochemical selectivity since release can only be measured at the site of the probe. EEDQ alkylation offers advantages over other methods of measuring receptor occupancy *in vivo* including positron emission tomography (PET) scans which are not readily available for small animals and are very expensive (Saller et al., 1989). *In vivo* radioligand labelling is also subject to problems with specificity, accessibility and metabolism *en route* to the brain. In the present investigations, EEDQ will be used to elucidate the functioning DA and 5-HT systems after chronic cocaine administration in the search for mechanisms of behavioural sensitization.

1.6. THESIS OBJECTIVES

The primary objective of the research in this thesis is to increase understanding of the mechanisms of behavioural sensitization and tolerance. This objective will be achieved through the use of receptor alkylation with EEDQ which will be used to investigate:

1) the effects of chronic cocaine on DA receptor occupation
    a) as it correlates with spontaneous behavioural activity in a continuous infusion paradigm and
    b) when delivered continuously to produce behavioural tolerance and

intermittently to produce sensitization;

2) the role of 5-HT in mediating behavioural sensitization and tolerance to cocaine.

1.7. REFERENCES

Akimoto, K., Hamamura, T. and Otsuki, S. (1989) Subchronic cocaine treatment enhances cocaine-induced dopamine efflux: studies by *in vivo* intracerebral dialysis. *Brain Res. 490*: 339-344.

Akimoto, K., Hamamura, T., Kazahaya, Y., Akiyama, K. and Otsuki, S. (1990) Enhanced extracellular dopamine levels may be the fundamental neuropharmacological basis of cross-behavioural sensitization between methamphetamine and cocaine - an *in vivo* dialysis study in freely moving rats. *Brain Res. 507*: 344-346.

Angrist, B. (1983) Psychoses induced by central nervous system stimulants and related drugs, In Creese, I. (Ed), *Stimulants: neurochemical, behavioural, and clinical perspectives*, Raven Press, New York, pp. 1-30.

Antelman, S.M. and Chiodo, L.A. (1981) Dopamine autoreceptor subsensitivity: a mechanism common to the treatment of depression and the indication of amphetamine psychosis. *Biol. Psychiat., 16:*717-727.

Argiolas, A, Melis, M.R., Fadda, F. and Gessa, G.L. (1982) Evidence for dopamine autoreceptors controlling dopamine synthesis in the substantia nigra. *Brain Res., 234*:177-181.

Barr, G.A., Sharpless, N.S., Cooper, S., Schiff, S.R., Paredes, W. and Bridger, W.H. (1983) Classical conditioning, decay and extinction of cocaine-induced hyperactivity and stereotypy. *Life Sci. 33:*1341-1351.

Baumann, P.A. and Maitre, L. (1976) Is drug inhibition of dopamine uptake a misinterpretation of in vitro experiments? *Nature, 264:*789-790.

Belleau, B., DiTullio V. and Godin, D. (1969) The mechanism of irreversible adrenergic blockade by N-carbethoxydihydroquinolines--model studies with typical serine hydrolases. *Biochem. Pharmacol., 18:* 1039-1044.

Belleau, B. and Malek, G. (1968) A new convenient reagent for peptide syntheses. *J. Am. Chem. Soc., 90:6,* 1651-1652.

Belleau, B., Martel, R., Lacasse G., Menard, M., Weinberg, N.L. and Perron, Y.G. (1968) N-carboxylic acid esters of 1,2- and 1,4-dihydroquinolines. A new class of irreversible inactivators of the catecholamine α receptors and potent central nervous system depressants. *J. Am. Chem. Soc., 90:* 823-824.

Besson, M.-J., Cheramy, A., Gauchy, C. and Musacchio, J. (1973) Effects of some psychotropic drugs on tyrosine hydroxylase activity in different structures of the rat brain. *Eur. J. Pharmacol., 22:*181-186.

Beninger, R.J. and Herz, R.S. (1986) Pimozide blocks establishment but not expression of cocaine-produced environment-specific conditioning. *Life Sci. 38*:1425-1431.

Bleich, A., Brown, S.L., Kahm, R. and Van Praag, H.M. (1988) The role of serotonin in schizophrenia. *Schizophrenia Bull., 14:*297-315.

Brock, J.W., Ng, J.P., and Justice, J.B. Jr. (1990) Effect of chronic cocaine on dopamine synthesis in the nucleus accumbens as determined by microdialysis perfusion with NSD-1015. *Neurosci. Lett., 117*:234-239.

Camp, D.M. and Robinson, T.E. (1985) Sex differences in an animal model of amphetamine psychosis. *Soc. Neurosci. Abstr., 11:*550.

Carboni, E., Imperato, A., Perezzani, L. and Di Chiara, G. (1989) Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. *Neurosci., 28:*653-661.

Carlsson, A. and Lindquist, M. (1963) Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacol. Toxicol. (Copenh.), 20:*140.

Castaneda, E., Becker, J.B., and Robinson, T.E. (1988) The long-term effects of amphetamine treatment in vivo on amphetamine, KCl and electrical stimulation evoked striatal dopamine release in vitro, *Life Sci.*, *42*: 2447-2456.

Connell, P.H. (1958) *Amphetamine psychosis.* Maudsley Monographs No. 5. Oxford University Press, London.

Cox, T.C., Jacobs, M.R., Leblanc, A.E. and Marshman, J.A. (1983) *Drugs and drug abuse*. Addiction Research Foundation, Toronto.

Cunningham, K.A., Paris, J.M. and Goeders, N.E. (1992a) Chronic cocaine enhances serotonin autoregulation and serotonin uptake binding. *Synapse, 11:*112-123.

Cunningham, K.A., Paris, J.M. and Goeders, N.E. (1992b) Serotonin neurotransmission in cocaine sensitization. *Ann. N.Y. Acad. Sci., 654:*117-127.

Dougherty, G.G. Jr. and Ellinwood, E.H. Jr. (1981) Chronic d-amphetamine in nucleus accumbens: lack of tolerance or reverse tolerance of locomotor activity. *Life Sci., 28:*2295-2298.

Drew, K.L. and Glick, S.D. (1988) Characterization of the associative nature of sensitization to amphetamine-induced circling behavior and of the environment dependent placebo-like response. *Psychopharmacology 95:*482-487.

Dwoskin, L.P, Peris, J., Yasuda, R., Philpott, K. and Zahniser, N.R. (1988) Repeated cocaine administration results in supersensitivity of striatal D2 dopamine autoreceptors to pergolide. *Life Sci., 42:*255-262.

Einhorn, L.C., Johansen, P.A. and White, F.J. (1988) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J. Neurosci., 8:*100-112.

Ellinwood, E.H. Jr., Kilbey, M.M., Castellani, S. and Khoury, C. (1977) Amygdala hyperspindling and seizures induced by cocaine. In: Ellinwood E.H. and Kilbey, M.M. (eds) *Advances in behavioural biology, vol. 21. Cocaine and other stimulants.* Plenum Press, New York, pp.303-326.

Ellison, G.D. (1991) Animal models of hallucinations. In: Boulton A.A., Baker G.B., Martin-Iverson M.T. (eds) *Neuromethods, Vol 18: Animal models in psychiatry I.* The Humana Press Inc., Totowa, N.J. pp 151-195.

Fischman, M.W. (1984) The behavioral pharmacology of cocaine in humans. *In:* Grabowski, J. (Ed) Cocaine: Pharmacology, effects, and treatment off abuse. National Institute on Drug Abuse Research Monograph #50, US Government Printing Office, Washington DC, pp.72-91.

Fitzgerald, J.L. and Reid, J.J. (1991) Chronic cocaine treatment does not alter rat striatal D2 autoreceptor sensitivity to pergolide. *Brain Res., 541:*327-333.

Friedman, E., Gershon, S. and Rotrosen J. (1975) Effects of cocaine treatment on the turnover of 5-hydroxytryptamine in the rat brain. *Br. J. Pharmacol., 54:*61-

Galloway, M.P. (1990) Regulation of dopamine and serotonin synthesis by acute administration of cocaine. *Synapse, 6:*63-72.

Galloway, M.P. (1992) Neuropharmacology of cocaine: effects on dopamine and serotonin systems. In J.M. Lakoski, M.P. Galloway and F.J. White (eds.), *Cocaine: pharmacology, physiology, and clinical strategies,* CRC Press, Boca Raton, FL, pp. 163-189.

Gerfen, C.R. and Young, W.S. (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both paatch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Res., 460:*161-167.

Goeders, N.E. (1990). The effects of chronic cocaine administration on brain neurotransmitter receptors. *Drug Devel. Res., 20:*349-357.

Goodman, L.S. and Gillman, A. (1965) *The pharmacological basis of therapeutics, 3rd ed.*, Macmillan, New York.

Goudie, A.J. and Emmett-Oglesby, M.W. (1989) Tolerance and sensitization: an overview. In: Goudie, A.J. and Emmett-Oglesby, M.W. (eds), Psychotropic drugs: tolerance and senstization, Humana Press, Clifton, NJ., pp.1-14.

Hamblin, M.W. and Creese, I. (1983) Behavioural and radioligand binding evidence for irreversible dopamine receptor blockade by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. *Life Sci., 32:*2247-2255.

Heikkila, R.E., Orlansky, H. and Cohen, G. (1975) Studies on the distinction between uptake inhibition and release of ^{3}H dopamine in rat brain tissue slices. *Biochem.*

*Pharmacol., 24:*84-852.

Henry, D.J., Greene, M.A., and White, F.J. (1989) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Repeated administration. *J. Pharmacol. Exp. Ther., 251:*833-839.

Henry, D.J. and White, F.J. (1991) Repeated cocaine administration causes persistent enhancement of D1 dopamine receptor supersensitivity within the rat nucleus accumbens. *J. Pharmacol. Exper. Ther., 258*:882-890.

Hinson, R.E., and Poulos, C.X. (1981) Sensitization of the behavioral effects of cocaine: modification by Pavlovian conditioning. *Pharmacol. Biochem. Behav. 15*:559-562.

Hitzemann, R.J., Tseng, L.F., Hitzemann, B.A., Samphath-Khanna, S. and Loh, H.H. (1977) Effects of withdrawal from chronic amphetamine intoxication on exploratory and stereotyped behaviours in the rat. *Psychopharmacol., 54:*295-302.

Hitzemann, R.J., Wu, J., Hom, D. and Loh, H. (1980) Brain locations controlling the behavioural effects of chronic amphetamine intoxication. *Psychopharmacol., 72:*93-101.

Hurd, Y.L. and Ungerstedt, U. (1989) Cocaine: an *in vivo* microdialysis evaluation of its acute action on dopaminergic transmission in rat striatum. *Synapse, 3:*48-54.

Hurd, Y.L., Weiss, F., Koob, G.F. and Ungerstedt, N (1989) Cocaine reinforcement and extracellular dopamine overflow in rat nucleus accumbens: an *in vivo* microdialysis study. *Brain Res. 498*: 199-203.

Johansson, C.-E., and Fischman, M.W. (1989) The pharmacology of cocaine related to its abuse. *Pharmacol. Rev., 41:*3-51.

Joyce, E.M. and Iversen, S.D. (1984) Dissociable effects of 6-OHDA-induced lesions of neostriatum on anorexia, locomotor activity and stereotypy: the role of behavioral competition. *Psychopharmacol., 83:*363-366.

Kalivas, P.W. and Duffy, P. (1988) Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release. *J. Neurochem., 50*:1498-1504.

Kalivas, P.W. and Duffy, P. (1989) Similar effects of daily cocaine and stress on mesocorticolimbic dopamine neurotransmission in the rat. *Biol. Psychiat., 25:*913-928.

Kalivas, P.W. and Duffy, P. (1990) The effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse, 5:*48-58.

Kalivas, P.W. and Duffy, P. (1993) Time course of extracellular dopamine and behavioural sensitization to cocaine. I. dopamine axon terminals. *J. Neurosci. 13:* 266-275.

Kalivas, P.W. and Weber, B. (1988) Amphetamine injection into the A10 dopamine region sensitizes rats to peripheral amphetamine, and cocaine. *J. Pharmacol. Exp. Ther., 245:*1095-1102.

Kalivas, P.W., Duffy, P., Dumars, L.A. and Skinner, C. (1988) The behavioural and neurochemical effects of acute and daily cocaine administration in rats. *J. Pharmacol. Exp. Ther., 245:*485-492.

Kalivas, P.W., Sorg, B.A. and Hooks, M.S. (1993) The pharmacology and neural circuitry of sensitization to psychostimulants. *Behav. Pharmacol., 4:*315-334.

Kelly, P.H. and Iversen, S.D. (1975) Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant-induced locomotor activity in rats. *Eur. J. Pharmacol., 40:*45-56.

Kennedy, L.T. and Hanbauer, I. (1983) Sodium-sensitive cocaine binding to rat striatal membrane: possible relationship to dopamine uptake sites. *J. Neurochem., 41:*172-178.

Kilbey, M.M. and Ellinwood, E.H. (1977a) Chronic administration of stimulant drugs: response modification. In: Ellinwood E.H. and Kilbey, M.M. (eds) *Advances in behavioural biology, vol. 21. Cocaine and other stimulants.* Plenum Press, New York, pp.409-430.

Kilbey, M.M. and Ellinwood, E.H. (1977b) The effect of age-related factors on behaviour induced by cocaine. *Life Sci., 20:*1847-1854.

King, G.R., Joyner, C., Lee, T. and Ellinwood, E.H. (1992) Intermittent and continuous cocaine administration: residual behavioral states during withdrawal. *Pharmacol. Biochem. Behav. 43:*243-248.

King, G.R., Kuhn, C., and Ellinwood, E.H. (1993) Dopamine efflux during withdrawal from continuous or intermittent cocaine. *Psychopharmacol., 111*: 179-184.

Kiyatkin, E.A. (1993) Cocaine enhances the changes in extracellular dopamine in nucleus accumbens associated with reinforcing stimuli: A high speed chronoamperometric study in freely moving rats. *Eur. J. Neurosci., 5:*284-291.

Klawans, H.L. and Margolin, D. (1975) Amphetamine-induced dopaminergic hypersensitivity in guinea pigs. *Arch. Gen. Psychiat., 32:*725-732.

Klein, W.L., Sullivan, J., Skorupa, A. and Aguilar, J.S. (1989) Plasticity of neuronal receptors. *FASEB J., 3:*2132-2140.

Kleven, M.S., Perry, B.D., Woolverton, W.L. and Seiden, L.S. (1990) Effects of repeated injections of cocaine on D1 and D2 dopamine receptors in rat brain. *Brain Res., 532:*265-270.

Kleven, M.S., Woolverton, W.L. and Seiden, L.S. (1988) Lack of long-term monoamine depletions following repeated or continuous exposure to cocaine. *Brain Res. Bull., 21:*233-237.

Kuczenski, R. and Leith, N.J. (1981) Chronic amphetamine: is dopamine a link in or mediator of the development of tolerance and reverse tolerance? *Pharmacol. Biochem. Behav., 15:*405-413.

Kuczenski, R. and Segal, D. (1989) Concomitant characterization of behavioural and striatal neurotransmitter response to amphetamine using *in vivo* microdialysis. *J. Neurosci., 9:*2051-2065.

Kuczenski, R., Segal, D.S. Weinberger, S.B. and Browne, R.G. (1982) Evidence that behavioural augmentation following repeated amphetamine adminstration does not involve peripheral mechanisms. *Pharmacol. Biochem. Behav. 17:*547-553.

Lakoski, J.M. and Cunningham, K.A. (1988) Cocaine interaction with central monoaminergic systems: electrophysiological approaches. *Trend Pharmacol. Sci., 9:*177-180.

Langer, S.Z. and Enero, M. (1974) The potentiation of responses to adrenergic nerve stimulation in the presence of cocaine: Its relationship to the metabolic fate of released norepinephrine. *J. Pharmacol. Exp. Ther., 191:*431-443.

Le, A.D. and Khanna, J.M. (1989) Dispositional mechanisms in drug tolerance and sensitization. In: Goudie, A.J. and Emmett-Oglesby, M.W. (eds), *Psychoactive drugs: Tolerance and sensitization*, Humana Press, Clifton, pp.17-114.

Lin-Chu, G., Robinson, T.E. and Becker, J.B. (1985) Sensitization of rotational behaviour produced by a single exposure to cocaine. *Pharmacol. Biochem. Behav., 22:*901-903.

Ljundberg, T. and Ungerstedt, U. (1985) A rapid and simple behavioural screening method for simultaneous assessment of limbic and striatal blocking effects of neuroleptic drugs. *Pharmacol. Biochem. Behav., 23:*479-485.

Lyon, M. and Robbins, R. (1975) The action of central nervous system stimulant drugs; a general theory concerning amphetamine effects. In:Essman, W.B. and Valzelli, L. (eds) *Current developments in psychopharmacology, Vol 2.*, Spectrum Publications, New York, pp.79-163.

Martin-Iverson, M.T. (1991a) An animal model of stimulant-induced psychoses. In: Boulton, A.A., Baker, G.B. and Martin-Iverson, M.T. (eds) *Neuromethods, Vol 19, Animal models in psychiatry I.* Humana Press, New Jersey, pp. 103-149.

Martin-Iverson, M.T. (1991b) Chronic treatment with D1 and D2 dopamine receptor agonists: combined treatments interact to differentially affect brain levels of monoamines. *Naunyn-Schmiedeberg's Arch. Pharmacol., 344:*281-285.

Martin-Iverson, M.T., DiLullo, S.L. and Reimer, A.R. (1993) Nimodipine and haloperidol interactions in amphetamine- and cocaine-conditioned behaviours.

*Drugs in Development, Vol 2:*417-433.

Martin-Iverson, M.T., Iversen, S.D. and Stahl, S.M. (1988a) Long-term motor stimulant effects of (+)-4-propyl-9-hydroxynaphthoxazine (PHNO), a dopamine D2 receptor agonist: interactions with a dopamine D1 receptor antagonist and agonist. *Eur. J. Pharmacol., 149:*25-31.

Martin-Iverson, M.T., Stahl, S.M. and Iversen, S.D. (1988b) Chronic administration of a selective dopamine D2 agonist: factors determining behavioural tolerance and sensitization. *Psychopharmacol., 95*: 534-539.

Martin-Iverson, M.T., Stahl, S.M. and Iversen, S.D. (1987) Factors determining the behavioural consequences of continuous treatment with 4-propyl-9-hydroxynaphthoxazine, a selective dopamine D2 agonist. In: F.C. Rose (ed), *Parkinson's Disease: clinical and experimental advances.* J. Libbey & Co., London, pp.169-177.

Mattingly, B.A. and Gotsick, J.E. (1989) Conditioning and experiential factors affecting the development of sensitization to apomorphine. *Behav. Neurosci. 103*:1311-1317.

Meller, E., Helmer-Matyjek, R., Bohmaker, K., Adler, C.H., Friedhoff, A.J. and Goldstein, M. (1986) Receptor reserve at striatal dopamine autoreceptors: implications for selectivity of dopamine agonists. *Eur. J. Pharmacol., 123:* 311-314.

Nagy, J.I. and Fibiger, H.C. (1980) A striatal source of glutamic acid decarboxylase activity in the substantia nigra. *Brain Res., 187:*237-242.

Nelson, L.R. and Ellison, G. (1978) Enhanced stereotypies after repeated injections but not continuous amphetamines. *Neuropsychopharmacol., 17:*1081-1084

Nielsen, E.B. (1981) Rapid decline of stereotyped behaviour in rats during constant one week administration of amphetamine *via* implanted ALZET osmotic minipumps. *Pharmacol. Biochem. Behav., 15:*161-165.

Nishikawa, T., Mataga, N., Takashima, M. and Toru, M. (1983) Behavioural sensitization and relative hyperresponsiveness of striatal and limbic dopaminergic neurons after repeated methamphetamine treatment. *Eur. J. Pharmacol., 88:*195-203.

Parsons, L.H. and Justice, J.B. Jr. (1993a) Quantitative approaches to *in vivo* brain microdialysis. *Critical Rev. Neurobiol.,* in press.

Parsons, L.H. and Justice, J.B. Jr. (1993b) Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area and dorsal raphe nucleus following repeated cocaine admininstration. *J. Neurochem.,61:*1611-1619.

Parsons, L.H., Smith, A.D., and Justice, J.B. Jr. (1991) Basal extracellular dopamine is decreased in the rat nucleus accumbens during abstinence from chronic cocaine. *Synapse, 9:*60-65.

Peris, J., Boyson, S.J., Cass, W.A., Curella, P., Dwoskin, L.P., Larson, G. Lin, L., Yasuda, R.P. and Zahniser, N.R. (1990) Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. *J. Pharmacol. Exp. Ther., 253*:38-44.

Peris, J. and Zahniser, N.R. (1987) One injection of cocaine produces a long-lasting increase in ^{3}[H]-dopamine release. *Pharmacol. Biochem. Behav., 29:*239-242.

Pettit, H.O., Pan, H.-T., Parson, L.H. and Justice, J.B. Jr. (1990) Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration, *J. Neurochem.*, *55*: 798-804.

Phillips, A.G., Broekkamp, C.L.E. and Fibiger, H.C. (1983) Strategies for studying the neurochemical substrates of drug reinforcement in rodents. *Prog. Neuropsychpharmacol. Biol. Psychiat.*, *7*:585.

Pickens, R. and Thompson, T. (1968) Cocaine-reinforced behaviour in rats:effects of reinforcement magnitude and fixed-ratio size. *J. Pharmacol. Exp. Ther.*, *161*:122-129.

Pitts, D.K. and Marwah, J. (1986) Electrophysiological effects of cocaine on central monoaminergic neurons. *Eur. J. Pharmacol.*, *131*:95-98.

Pitts, D.K. and Marwah, J. (1987) Cocaine modulation of central monoaminergic neurotransmission. *Pharmacol. Biochem. Behav.*, *26*:453-461.

Post, R.M. (1980) Intermittent versus continuous stimulation: effect of time interval on the development of sensitization or tolerance, *Life Sci.*, *26*: 1275-1282

Post, R.M., Lockfield, A., Squillace, K.M. and Contel, N.R. (1981) Drug-environment interaction: context dependency of cocaine-induced sensitization. *Life Sci.* *28*:755-760.

Post, R.M., Weiss, S.R.B., Fontana, D. and Pert, A. (1992) Conditioned sensitization to the psychomotor stimulant cocaine. *Annals NY Acad. Sci.*, *654*:386-399.

Post, R. M. and Contel, N.R. (1983) Human and animal studies of cocaine: implications for development of behavioural pathology. In: Creese I (ed) *Stimulants:*

neurochemical, behavioral and clinical perspectives. Raven Press, New York, pp 169-203.

Post, R.M. and Rose, H. (1976) Increasing effects of repetitive cocaine administration in the rat. *Nature, 260:*731-732.

Post, R.M. and Weiss, S.R.B. (1988) Sensitization and kindling: implications for the evolution of psychiatric symptomatology. In: Kalivas, P.W. and Barnes, C.D. (eds) *Sensitization in the nervous system*, Telford Press, Caldwell, NJ, pp.257-292.

Pradhan, S.N., Battacharyya, A.K. and Pradhan, S. (1978) Serotonergic manipulation of the behavioural effects of cocaine in rats. *Comm. Psychopharmacol., 2:*481-486.

Reimer, A.R. and Martin-Iverson, M.T. (1994) Nimodipine and haloperidol attenuate behavioural sensitization to cocaine but only nimodipine blocks the establishment of conditioned locomotion induced by cocaine, *Psychopharmacol. 113:* 404-410.

Reith, M.E.A. (1986) Effect of repeated administration of various doses of cocaine and WIN 35,065-2 on locomotor behaviour of mice. *Eur. J. Pharmacol.*, 130:*65-72.*

Reith, M.E.A. (1992) Central and peripheral cocaine binding sites. In J.M. Lakoski, M.P. Galloway and F.J. White (eds.), *Cocaine: Pharmacology, physiology and clinical strategies,* CRC Press, Boca Raton, FL, pp. 203-227.

Reith, M.E.A., Sershen, H., Allen, D.L. and Lajtha, A. (1983) A portion of [^{3}H]cocaine binding in brain is associated with serotonergic neurons. *Mol. Pharmacol., 23:*600-606.

Reith, M.E.A., Benuck, M. and Lajtha, A. (1987) Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. *J. Pharmacol. Exper. Ther., 243:*281-287.

Roberts, D.C.S., Corcoran, M.E., and Fibiger, H.C. (1977) On the role of ascending catecholamine systems in intravenous self-administration of cocaine. *Pharmacol. Biochem. Behav., 6:*615.

Roberts, D.C.S., Koob, G.F., Klonoff, P. and Fibiger, H.C. (1980) Extinction and recovery of cocaine self-administration following 6-OHDA lesions of the nucleus accumbens. *Pharmacol. Biochem. Behav., 12:*781-787.

Robinson, T.E. (1991) The neurobiology of amphetamine psychosis: evidence from studies with an animal model. In: Nakazawa, T. (ed) *Taniguchi symposia on brain sciences, Vol 14, Biological basis of schizophrenic disorders.* Japan Scientific Societies Press, Tokyo.

Robinson, T.E. and Becker, J.B. (1982) Behavioural sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro, *Eur. J. Pharmacol., 85*: 253-254.

Robinson, T.E. and Becker, J.B. (1986) Enduring changes in brain and behaviour produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis, *Brain Res. Rev., 11*: 157-198.

Robinson, T.E., Becker, J.B. and Presty, S.K. (1982) Long-term facilitation of amphetamine-induced rotational behaviour and striatal dopamine release produced by a single exposure to amphetamine: sex differences, *Brain Res., 253*: 231-241.

Robinson, T.E., Jurson, P.A., Bennett, J.A. and Bentgen, K.M. (1988) Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)amphetamine: a microdialysis study in freely moving rats. *Brain Research, 462*: 211-222.

Robinson, T.E., Yew, J., Paulson, P.E. and Camp, D.M. (1990) The long-term effects of neurotoxic doses of methamphetamine on the extracellular concentration of dopamine measured with microdialysis in striatum. *Neurosci. Lett. 110:*193-198.

Ross, S.B. and Renyi, A.L. (1967) Inhibition of the uptake of tritiated catecholamines by antidepressant and related agents. *Eur. J. Pharmacol., 2:*181-186.

Ross, S.B. and Renyi, A.L. (1969) Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. *Eur. J. Pharmacol., 7:*270-277.

Ryan, L.J., Martone, M.E., Linder, J.C., and Groves, P.M. (1988) Cocaine, in contrast to d-amphetamine, does not cause axonal terminal degeneration in neostriatum and agranular frontal cortex of long-evans rats. *Life Sci., 43:* 1403-1409.

Saller, C.F., Kreamer, L.D., Adamovage, L.A., and Salama, A.I. (1989) Dopamine receptor occupancy *in vivo:* measurement using N-ethoxycarbonyl-2-ethoxy-1,2-dihyroquinoline (EEDQ). *Life Sci., 45:* 917-929.

Savasta, M., Dubois, A., Benavides, J. and Scatton, B. (1986) Different neuronal location of [^{3}H]SCH 23390 binding sites in pars reticulata and pars compacta of the substantia nigra in the rat. *Neurosci. Lett., 72*:265-271.

Scheel-Kruger, J. (1971). Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain. *Eur. J. Pharmacol., 14:*47-59.

Scheel-Kruger, J., Braestrup, C., Nielson, M., Golembiowska, K., and Mogilnicka, E. (1977) Cocaine: discussion on the role of dopamine in the biochemical mechanisms of action. In: Ellinwood Jr., E.H. and Kilbey, M.M. (Eds). *Cocaine and other stimulants*. Plenum Press, New York, pp.373-408.

Schoemaker, H., Pimoule, C., Arbilla, S., Scatton, B., Jovoy-Agid, F. and Langer, S.Z. (1985) Sodium dependent [^{3}H]cocaine binding associated with dopamine uptake sites in the rat striatum and human putamen decrease after dopaminergic denervation and in Parkinson's Disease. *Naunyn-Schmiedeberg's Arch. Pharmacol., 329:*227.

Segal, D.S. and Kuczenski, R. (1992a) *In vivo* microdialysis reveals a diminished amphetamine-induced dopamine response corresponding to behavioural sensitization produced by repeated amphetamine pretreatments. *Brain Res. 571*: 330-337.

Segal, D.S. and Kuczenski, R. (1992b) Repeated cocaine administration induces behavioural sensitization and corresponding decreased extracellular dopamine responses in caudate and accumbens. *Brain Res. 577*: 351-355.

Segal, D.S., and Schuckit, M.A. (1983) Animal models of stimulant induced psychosis. In: Creese I (ed) *Stimulants: neurochemical, behavioral and clinical perspectives.* Raven Press, New York, pp 131-168.

Shuster, L., Yu, G. and Bates, A. (1977) Sensitization to cocaine stimulation in mice. *Psychopharmacol., 52:*185-190.

Snoddy, A.M. and Tessel, R.E. (1985). Prazosin: effect on psychomotor-stimulant cues and locomotor activity in mice. *Eur. J. Pharmacol.,116:*221-228.

Stewart, J. and Vezina, P. (1989) Microinjections of SCH 23390 into the ventral tegmental area and substantia nigra pars reticulata attenuate the development of sensitization to the locomotor activating effects of systemic amphetamine, *Brain Res.*, *495:* 401-406.

Stewart, J. and Vezina, P. (1991) Extinction procedures abolish conditioned stimulus control but spare sensitized responding to amphetamine. *Behav. Pharmacol. 2:*65-71.

Stripling, J.S. and Ellinwood, E.H. Jr. (1977) Potentiation of the behavioural and convulsant effects of cocaine by chronic administration in the rat. *Pharmacol. Biochem. Behav., 6:*571-579.

Taylor D. and Ho, B.T. (1977) Neurochemical effects of cocaine following acute and repeated injection. *J. Neurosci. Res , 3:*95-101.

Tilson, H.A., and Rech, R.H. (1973) Conditioned drug effects and absence of tolerance to d-amphetamine induced motor activity. *Pharmacol. Biochem. Behav. 1*:149-153.

Vezina, P. (1993) Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: an *in vivo* microdialysis study in the rat. *Brain Res., 605:*332-337.

Vezina, P. and Stewart, J. (1990) Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. *Brain Res., 516:*99-106.

Weiss, F. Paulus, M.P., Lorang, M.T. and Koob, G.F. (1992) Increases in extracellular dopamine in the nucleus accumbens by cocaine are inversely related to basal levels: Effects of acute and repeated administration. *J. Neurosci., 12:*4372-4380.

Weiss, S.R.B., Post, R.M., Szele, F., Woodward, R. and Nierenberg, J. (1989) Chronic carbamazepine inhibits the development of local-anesthetic seizures kindled by cocaine and lidocaine. *Brain Res., 497:*72.

White, F.J., Wachtel, S.R., Johansen, P.A., and Einhorn, L.C. (1987) Electrophysiological studies of the rat mesoaccumbens dopamine system: focus on dopamine receptor subtypes, interactions and the effects of cocaine. In: Chiodo, L.A. and Freeman, A.S. (eds) *Neurophysiology of dopaminergic systems-current status and clinical perspectives.* Lakeshore Publishing, Detroit, pp. 317.

White, F.J., Henry, D.J., Hu, X.T., Jeziorski, M. and Ackerman, J.M. (1992) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system. In: Lakoski, J.M., Galloway, M.P. and White, F.J. (eds) *Cocaine: pharmacology, physiology and clinical strategies,*CRC Press, Boca Raton, Fl., pp.261-294.

Wise, R.A. (1984) Neural mechanisms of the reinforcing action of cocaine. *NIDA Res. Monograph, 50:*15.

Wood, W.G., Schreiber, H.L., Villescas, R., and Carlson, R.H. (1977) Effects of prior experience and 'functional disturbance' on acute and chronic tolerance to methylphenidate. *Psychopharmacol., 51,:*165-168.

Yi, S.J. and Johnson, K.M. (1990) Effects of acute and chronic administration of cocaine on striatal uptake, compartmentalization and release of [^{3}H]dopamine. *Neuropharmacol., 29:*475-486.

CHAPTER 2. EEDQ AS A TOOL FOR ASSESSING RECEPTOR FUNCTION: A DOSE-RESPONSE ANALYSIS WITH COCAINE

2.1 INTRODUCTION

The use of agents that irreversibly bind to neurotransmitter receptors as tools for investigating receptor function has increased in recent years, particularly due to the discovery of the compound N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Originally developed as a peptide-coupling agent, EEDQ was subsequently found to have CNS depressant properties in conjunction with irreversible α-adrenergic receptor blockade (Belleau et al., 1968). Hamblin and Creese (1983) later extended the research to include DA receptor inactivation after peripheral administration of EEDQ. After a 6 mg/kg dose of EEDQ, both D1 and D2 DA receptor binding sites were reduced by approximately 75% in rat striatum. This receptor inactivation was accompanied by catalepsy and attenuated apomorphine-induced stereotypy. EEDQ has also been shown to inhibit both amphetamine-induced stereotypy and conditioned avoidance behaviour (Martel et al., 1969), further demonstrating its neuroleptic-like effects.

The use of EEDQ as a tool for studying DA receptor function was later extended to include selective inactivation of specific receptor subtypes as a pharmacological lesion. Meller et al. (1985) pretreated animals with the selective D2 antagonist sulpiride to protect those sites from EEDQ inactivation. Similarly, SCH23390, a D1 receptor antagonist, led to the selective reduction of D2 binding sites by EEDQ. D2 autoreceptor reserve in rat striatum has also been assessed by selective protection from EEDQ inactivation (Meller et al., 1986). These protection experiments therefore permit for the study of specific receptor function and repopulation (Fuxe et al., 1987) and can provide an *in vivo* assessment of drug effects at various receptors.

The irreversible nature of the EEDQ inactivation is due to its ability to convert carboxyl groups into mixed carbonic anhydrides which are subject to nucleophillic attack by sterically accessible nucleophillic groups at the receptor site (Belleau et al., 1968, 1969; Belleau and Malek, 1968). The resultant formation of a covalent bond prevents further receptor occupation by any exogenous or endogenous ligand. *In vitro* binding assays therefore enable an *in vivo* assessment of receptors protected from EEDQ inactivation after prior treatment with a known agonist or antagonist. The purpose of the present experiment was to examine the occupation of both D1 and D2 DA receptors after administration of the DA uptake blocker cocaine. Since the use of EEDQ as a probe for determining the effects of cocaine on DA receptor occupation had not been reported previously, a dose-response of acute cocaine administration was combined with a range of doses of EEDQ comparable to those used previously in the literature. The results were used to determine an appropriate dose of EEDQ for future study with chronic cocaine administration.

2.2 METHODS

2.2.1. Animals

Male Sprague-Dawley rats (250-350 g) were obtained from the University of Alberta (Ellerslie) and housed in pairs with free access to food and water. Room temperature was kept at 22 ^{0}C and a 12 hour light/dark cycle (lights on at 0800 h) was maintained.

2.2.2. Drug Administration

After habituation to colony conditions, each rat was assigned to one of 12 possible drug groups, for a total of 96 rats. Cocaine hyrochloride (BDH) was delivered in doses of 0, 8.3 or 25 mg/kg followed 30 min later by a second injection with EEDQ (Sigma) at either 0, 5, 10 or 20 mg/kg. Both injections were given intraperitoneally (i.p.). Cocaine was dissolved in double distilled H_2O while EEDQ was mixed in a solution of ethanol, propylene glycol and water (2:1:2).

2.2.3. Tissue Collection

At 24 hours post-EEDQ, all subjects (n=94, since 2 rats died before completion) were decapitated by guillotine and the brains quickly removed for immersion in ice cold saline. For regional dissection, the brain was placed on a saline-soaked filter paper on a bed of ice. The dissection procedure involved removal of the brain meninges from the dorsal and ventral brain. The frontal lobes were dissected by a coronal slice approximately 5 mm posterior to the olfactory bulbs. The frontal cortex was subsequently separated from olfactory tubercle. The remainder of the brain was placed ventral side up where a second coronal section was removed approximately 2 mm posterior to the previous slice. This section was further dissected to remove the nucleus accumbens, located near the midline, and a portion of the neighbouring striatum. The exposed cortex on the rest of the brain was subsequently peeled back for removal of the remaining striatal tissue. This method allows for removal of both the head and tail of the caudate. All myelin was removed from the tissues. Left and right sides of each of these brain regions were placed immediately on dry ice and then stored at at -80 ^{0}C for further neurochemical analysis. For the present experiment, only the striatal and olfactory tubercle samples were analyzed.

2.2.4. DA Receptor Binding

Unilateral striatal tissue from each rat was homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.4, 40 vols). The homogenate was centrifuged for 17 min at 22000 rpm and 4 °C. The pellet was resuspended and recentrifuged twice using incubation buffer for the final wash. For D2 receptor binding, this Tris-HCl buffer consisted of 120 mM NaCl and 5 mM KCl, with the addition of 2 mM $CaCl_2$ and 1 mM $MgCl_2$ for D1 receptor binding. The final tissue concentration was 1 mg/ml, 0.1 ml of which was added to incubation tubes in duplicate (D1) or triplicate (D2) containing 100 μl [^{3}H]SCH23390 (D1 binding) or [^{3}H]spiperone (D2 binding). Ligand concentrations for the D1 assay were 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 nM while D2 ligand concentrations included 0.015, 0.0312, 0.0625, 0.125, 0.25 and 0.5 nM. A volume of 100 μl each of solutions of butaclamol (final concentration of 1 μM) and ketanserin (final concentration of 40 nM) were also included in order to assess nonspecific binding and prevent $5HT_2$ receptor binding, respectively. Incubation buffer was added to bring the volume up to 1 ml per tube. After incubating at room temperature for 1 h, the samples were immediately filtered through glass fibre filters (Whatman GF/B) and rinsed 3 times with 5 ml ice-cold buffer (appropriate to assay). The filters were added to scintillation vials and filled with 5 ml Ready Safe liquid scintillation cocktail (Beckman). Radioactivity was assessed for 10 min per vial in a liquid scintillation counter (Beckman LS 7500) equipped for DPM calculation.

2.2.5. Protein Determination

Protein concentrations in rat brain homogenates were assessed by the method of Lowry et al. (1951). To an aliquot of brain homogenate (50 μl), 750 μl distilled water and 200 μl of membrane digestor (1:1 1N sodium hydroxide:sodium deoxycholate) were added. This mixture was vortexed and incubated at room temperature for 10 min. Reagent A (1:1:100 1% cupric sulfate:2% sodium potassium tartrate:2% sodium carbonate) was subsequently added to the tubes which were left for 10 min. Finally, Folin reagent (1:1 2N Folin-Phenol:double distilled water; 500 μl) was added, the samples mixed and incubated for 30 min. A standard curve, using bovine serum albumin as protein standard, was run in parallel with the tissue samples. All samples were run through a Unicam SP1700 Ultraviolet/Visible Spectrophotometer (Pye-Unicam, Cambridge, UK) set at a wavelength of 660 nm. The Lowry program (McPherson, 1987) was used to determine the protein content per tube.

2.2.6. HPLC Analysis

Olfactory tubercle samples were subjected to high pressure liquid chromatography (HPLC) analysis with electrochemical detection for determination of DA and acidic metabolite levels (particularly homovanillic acid, HVA). This brain region was used for turnover analysis because no striatal tissue was available for this procedure after D1 and D2 binding assays. Tissue samples were homogenized in 10 volumes of 0.1N $HClO_4$ containing 0.05 mM ascorbic acid and 10 mg % EDTA. The homogenate was then transferred into 1.5 ml Eppendorf microfuge tubes and centrifuged at high speed for 2 minutes (MSE microfuge) . The supernatant was subsequently transferred to HPLC vials where a 20 μl aliquot was injected and detected on the HPLC system described below.

The mobile phase, pumped at a flow rate of 1.0 ml/min, was composed of 55 mM NaH_2PO_4, 0.73 mM sodium octyl sulfate, 0.37 mM disodium EDTA and 10% acetonitrile; the pH was adjusted to 3.0 with phosphoric acid. The HPLC apparatus consisted of a solvent delivery system (model 510, Waters Associates, Milford MA) with an automatic injector (WISP model 710B, Waters Associates). An Econosphere™ C_{18} reverse-phase column (4.61 x 250 mm, Applied Science Labs, Avondale, PA) was used to separate the compounds of interest. A precolumn of 4.6 mm x 30 mm packed with the same material as the analytical column was also used. The elutants were then detected by an electrochemical detector (model 460, Waters) with an applied voltage setting of 0.85V. The signal was subsequently recorded as a peak on an HP 3392A integrator/printer.

Standard callibration curves of known concentrations of the amines and acid metabolites of interest were run in parallel with the tissue samples each assay day. Linearity of these calibration curves was confirmed by correlation coefficients (r^2) of 0.99 or greater.

2.2.7. Statistics

Receptor D1 and D2 Bmax (maximum number of binding sites) and K_d (dissociation constant) values were obtained from the data using the equilibrium binding data analysis (EBDA/ LIGAND, McPherson, 1987) and, as for DA turnover ratios, was subjected to ANOVA with two factors, EEDQ (4 levels of 0, 5, 10 and 20 mg/kg) and cocaine (3 levels of 0, 8.3, 25 mg/kg). Bmax data was analyzed with receptor subtype as a within subject factor. Planned comparisons, where appropriate, were examined using multiple F tests (Kiess, 1989).

2.3. RESULTS

2.3.1. DA Receptor Binding

D1 binding results showed that all three doses of EEDQ (5, 10 and 20 mg/kg) significantly reduced receptor Bmax compared to untreated controls (Fig. 2-1). This decrease in D1 receptor density was similar across doses and represented a 54-65% loss of receptors. In those animals pre-treated with cocaine, a dose-dependent protection from EEDQ denaturation was observed. With 8.3 mg/kg cocaine, D1 receptors were significantly protected from EEDQ at the lowest dose, but not 10 or 20 mg/kg. With high dose cocaine pre-treatment however, protection from the higher doses of EEDQ was found but not at the 5 mg/kg dose. Similar findings were observed for D2 receptor binding. As shown in Fig. 2-2, all three doses of EEDQ significantly reduced receptor Bmax to at least 50% of controls with the high dose significantly more effective than the low and mid doses. Cocaine pre-treatment also served to protect D2 receptors from EEDQ denaturation in a similar fashion to that found with D1 binding. Again, low dose cocaine was sufficient to protect from low dose EEDQ but, unlike D1 receptors, also protected D2 receptors from further reduction by the 20 mg/kg dose of EEDQ. The high dose cocaine group showed higher D2 receptor levels at the mid and high EEDQ doses only, as seen for D1 receptor Bmax. These effects on Bmax are supported by ANOVA main effects of EEDQ

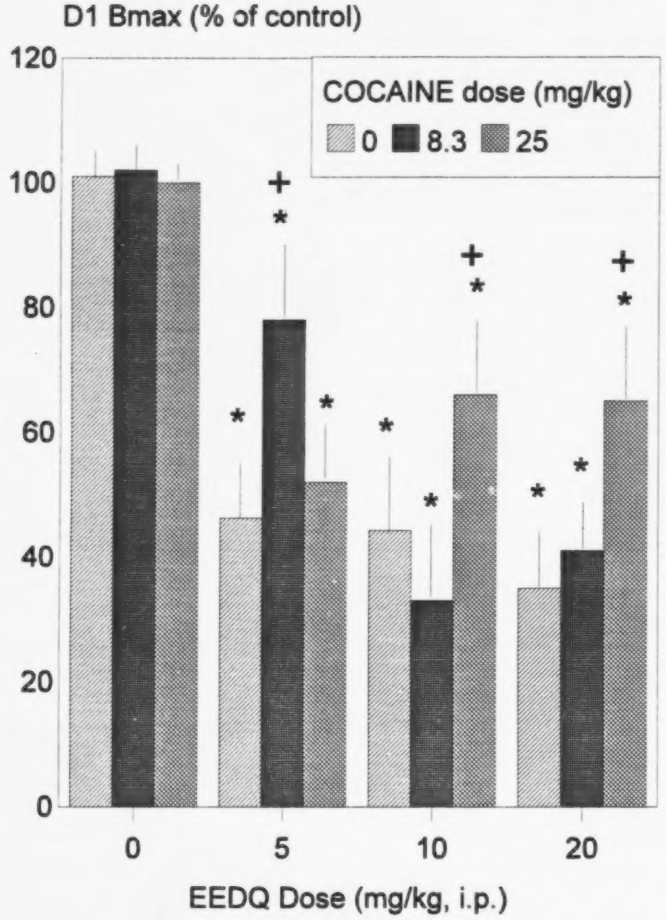

Fig. 2-1: Striatal D1 DA receptor density represented as mean percent of vehicle control (±SEM) after 5, 10 or 20 mg/kg EEDQ. Control Bmax = 1104.68 fmol/mg protein, ±42.53 (SEM) . Pre-treatment with cocaine protected D1 receptors from EEDQ inactivation in a dose-dependent fashion. * $p < .05$ significantly different from 0 mg/kg EEDQ. + $p < .05$ significantly different from 0 mg/kg cocaine.

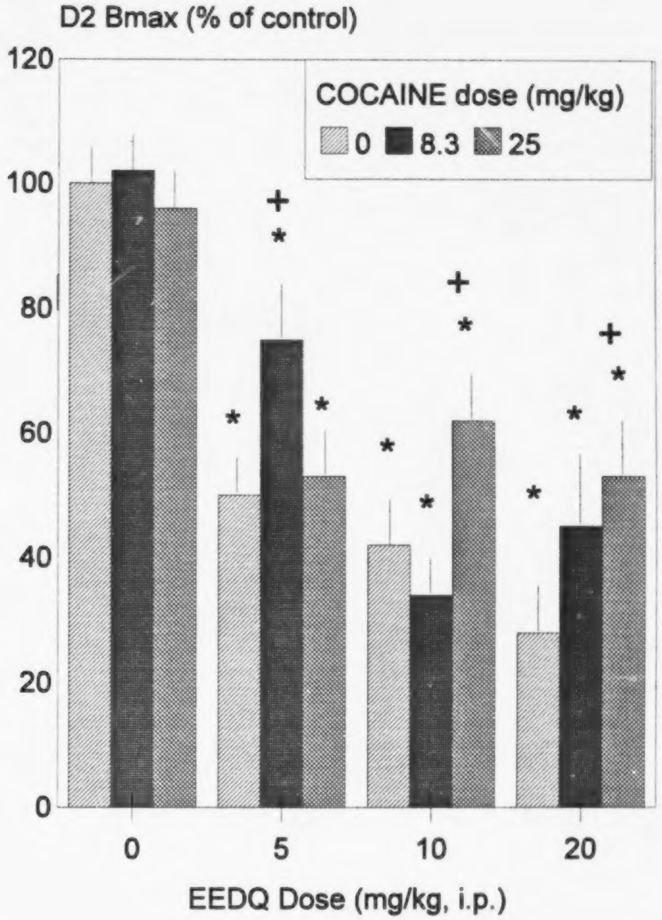

Fig. 2-2: D2 DA receptor occupancy in rat striatum represented as mean percent of vehicle control (± SEM) after several doses of EEDQ. Control Bmax = 375.41 fmol/mg protein ± 23.49 (SEM). Animals pre-treated with cocaine showed protection from EEDQ inactivation. * $p < .05$ significantly different from EEDQ control levels. + $p < .05$ significantly different from cocaine controls.

(F(3,78)=26.78; $p < .01$) and an EEDQ x cocaine interaction (F(6,78)=2.48; $p < .05$). No difference was found between D1 and D2 subtypes with ANOVA.

Table 2-1 shows K_d values for D1 and D2 receptor binding analysis for each dose of EEDQ. An overall main effect of EEDQ was found with ANOVA for D1 K_d values (F(3,80)=9.92, $p < .01$) but no significant effect on D2 affinity. Both the 10 and 20 mg/kg EEDQ doses significantly increased K_d, i.e. there was a decrease in the affinity of the D1 receptors for [^{3}H]SCH23390 *in vitro*. No significant change in K_d was found for either receptor at the 5 mg/kg level.

2.3.2. DA Turnover

HPLC techniques were used to measure DA and HVA levels in the olfactory tubercle. The ratio of HVA to DA in this area provides some information about the effect of EEDQ on synaptic DA metabolism since HVA is formed extraneuronally. As shown in Fig. 2-3, EEDQ treatment served to increase DA turnover at the 10 and 20 mg/kg doses but not at 5 mg/kg (F(3,82)=5.56, $p < .01$). This increase in DA turnover was attenuated by cocaine pre-treatment to a significant degree at the 10 mg/kg EEDQ dose.

2.4. DISCUSSION

The results of the present experiment support and extend previous reports of extensive DA receptor depletion produced by acute doses of the peptide-coupling agent EEDQ (Hamblin and Creese, 1983; Meller et al., 1985; Hess et al., 1987; Ekman and Eriksson, 1991). The reductions in both D1 and D2 receptor Bmax values found here range from 50-72% for doses between 5 and 20 mg/kg, respectively. These values fall

Table 2-1. K_d values (nM, ± SEM)for D1 and D2 receptors in rat striatum 24 hours after EEDQ administration. * $p < .05$ significantly different from control.

TREATMENT	n	^{3}[H]SCH23390 (D1)	^{3}[H]Spiperone (D2)
Control	8	0.197 ± .007	0.043 ± .002
EEDQ - 5 mg/kg	8	0.233 ± .014	0.049 ± .004
10 mg/kg	7	0.266 ± .027 *	0.047 ± .007
20 mg/kg	7	0.265 ± .018 *	0.035 ± .005

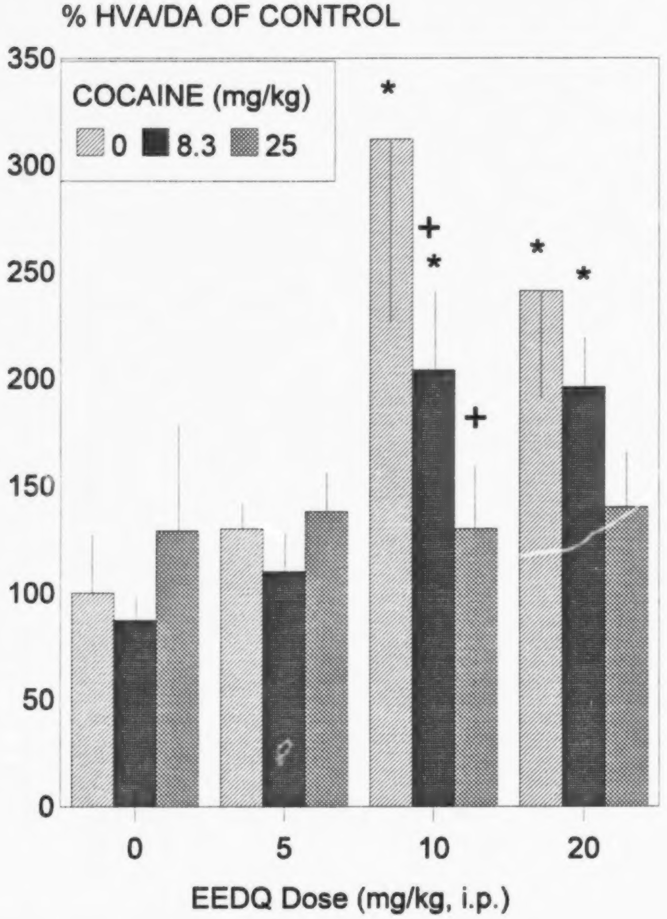

Fig. 2-3: Ratio of HVA to DA in olfactory tubercle after EEDQ alone or in combination with cocaine. Values are represented as mean percent of vehicle control (± SEM). Higher doses of EEDQ increased the turnover of DA to HVA relative to controls. * p<.05 significantly different from EEDQ controls. + p<.05 significantly different from cocaine controls.

in the same range as previous reports, although some studies have reported close to 90%reductions in striatal DA receptor density with the higher EEDQ doses (Battaglia et al., 1988; Ekman and Eriksson, 1991). Acute adminstration of the DA agonist cocaine offered significant protection for both D1 and D2 receptors from EEDQ inactivation in a dose-dependent fashion. The use of agonists and antagonists specific for the DA receptor as protective agents from EEDQ is well documented in the literature (Meller et al., 1985; Saller et al., 1989), but the effects of cocaine pretreatment in combination with several doses of EEDQ is a novel addition to the research in this field.

The action of cocaine to block DA reuptake and increase DA levels in the synapse prior to EEDQ administration can increase the occupation of DA receptors, presumably by DA, thereby protecting these receptors from EEDQ denaturation. The level of protection was found to vary with both the dose of cocaine given and the dose of EEDQ that followed. The low dose of cocaine protected D1 and D2 receptors from the lowest dose of EEDQ but was generally not sufficient to prevent the receptor depletion caused by the higher dose of EEDQ. The 25 mg/kg dose of cocaine, on the other hand, was successful in increasing receptor occupation at the mid and high doses of EEDQ but, interestingly, not at the lowest dose. This complex interaction between cocaine and EEDQ during the 24 hours post-treatment may be further understood when the effect of EEDQ on DA turnover is considered. High doses of EEDQ served to increase the levels of the extraneuronally produced metabolite HVA relative to DA in the olfactory tubercle. This finding gives an indication of EEDQ's effect at the DA synapse in the striatum based on the finding by Fuxe et al. (1987) that all brain regions are equally sensitive to EEDQ. A higher DA turnover may be expected if the number of DA receptors has been depleted, leaving more DA in the extracellular space available for metabolism. The higher the receptor depletion 24 hours later, the greater the turnover. Coupled with the probability that more autoreceptors and postsynaptic receptors involved in negative feedback to the DA neurons are denatured with the higher EEDQ doses, there is also a subsequent dampening of DA inhibition. Therefore, 24 hours post-EEDQ, more DA is available for breakdown into HVA. With prior cocaine treatment to enhance DA levels in the 30 minutes prior to EEDQ adminstration, more DA is also available for binding and protecting DA receptors. The finding that high dose cocaine fails to protect from a low dose of EEDQ may be due to the finding that higher doses of cocaine can lead to decreased impulse activity in DA cells (Einhorn et al., 1988; White, 1990). This may enable EEDQ to act at receptors with reduced protection from cocaine. In addition, the lower EEDQ dose may not be sufficient to dampen the DA inhibitory feedback systems, thereby resulting in less DA for metabolism or protection of remaining receptors within the 24 hours post-EEDQ.

In summary, the interactions between these two compounds given acutely is more complex when a higher dose of EEDQ is given. Considering that the original purpose of this study was to find an optimal dose of EEDQ to use as a tool for future studies on DA receptor occupation after chronic cocaine, the present data point to 5 mg/kg as most appropriate. This low dose does not complicate cocaine's effects on the DA system with respect to turnover, but is still sufficient for significant DA receptor depletion (without altering D1 receptor affinity) in the untreated brain.

2.5. REFERENCES

Battaglia, G., Norman, A.B. and Creese, I. (1988) Age-related differential recovery rates of rat striatal D1 dopamine receptors following irreversible inactivation. *Eur. J. Pharmacol., 145:* 281-290.

Belleau, B., DiTullio V. and Godin, D. (1969) The mechanism of irreversible adrenergic blockade by N-carbethoxydihydroquinolines--model studies with typical serine hydrolases. *Biochem. Pharmacol., 18:* 1039-1044.

Belleau, B. and Malek, G. (1968) A new convenient reagent for peptide syntheses. *J. Am. Chem. Soc., 90:6,* 1651-1652.

Belleau, B., Martel, R., Lacasse G., Menard, M., Weinberg, N.L. and Perron, Y.G. (1968) N-carboxylic acid esters of 1,2- and 1,4-dihydroquinolines. A new class of irreversible inactivators of the catecholamine α receptors and potent central nervous system depressants. *J. Am. Chem. Soc., 90:* 823-824.

Einhorn, L.C., Johanser, P.A. and White, F.J. (1988) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J. Neurosci., 8:* 100-112.

Ekman, A. and Eriksson, E. (1991) Pituitary and brain D2 receptor density measured *in vitro* and *in vivo* in EEDQ treated male rats. *Life Sci., 48:* 321-331.

Fuxe, K., Agnati, L.F., Pich, E.M., Meller, E. and Goldstein, M., (1987) Evidence for fast receptor turnover of D1 dopamine receptors in various forebrain regions of the rat. *Neurosci. Lett., 81:* 183-187.

Hamblin, M.W. and Creese, I. (1983) Behavioral and radioligand binding evidence for irreversible dopamine receptor blockade by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. *Life Sci., 32:* 2247-2255.

Hess, E.J., Battaglia, G., Norman, A.B., and Creese, I. (1987) Differential modification of striatal D1 dopamine receptors and effector moieties by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline *in vivo* and *in vitro*. *Mol. Pharmacol., 31:* 50-57.

Kiess, H.O. (1989) *Statistical Concepts for the Behavioral Sciences.* Allyn & Bacon, Needham Heights, MA., pp. 326-331.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurements with the Folin phenol reagent. *J. Biol. Chem., 193:*265-275.

Martel, R.R., Berman, R. and Belleau, B. (1969) Pharmacology of EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2dihyroquinoline). *Can. J. Physiol. Pharmacol. 47:* 909-912.

McPherson, G.A. (1987) Kinetic, EBDA, Ligand, Lowry. *A collection of radioligand binding analysis programs.* Elsevier-Biosoft, U.K.

Meller, E., Bohmaker, K., Goldstein, M., and Friedhoff, A.J. (1985) Inactivation of D1 and D2 dopamine receptors by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline *in vivo:* selective protection by neuroleptics. *J. Pharmacol. Exper. Ther. 233:3,* 656-662.

Meller, E., Helmer-Matyjek, R., Bohmaker, K., Adler, C.H., Friedhoff, A.J. and Goldstein, M. (1986) Receptor reserve at striatal dopamine autoreceptors: implications for selectivity of dopamine agonists. *Eur. J. Pharmacol., 123:* 311-

314.

Saller, C.F., Kreamer, L.D., Adamovage, L.A., and Salama, A.I. (1989) Dopamine receptor occupancy *in vivo:* measurement using N-ethoxycarbonyl-2-ethoxy-1,2-dihyroquinoline (EEDQ). *Life Sci., 45:* 917-929.

White, F.J. (1990) Electrophysiological basis of the reinforcing effects of cocaine. *Behav. Pharmacol., 1:* 303-315.

CHAPTER 3. DAY/NIGHT DIFFERENCES IN D1 BUT NOT D2 DA RECEPTOR PROTECTION FROM EEDQ DENATURATION IN RATS TREATED WITH CONTINUOUS COCAINE[1]

3.1. INTRODUCTION

Cocaine is thought to enhance dopamine (DA) neurotransmission in the brain by blocking the DA transporter responsible for removing most of the DA from the synaptic cleft, producing an increase in the extracellular concentration of DA (Hurd and Ungerstedt, 1989; Kalivas and Duffy, 1990; Pettit et al., 1990). This action is apparently responsible for the direct reinforcing properties of cocaine (Kuhar et al., 1991; Ritz et al., 1987). The increasing recreational use of this stimulant drug has prompted interest and concern regarding the long term effects of chronic cocaine. Study of the neural substrates of cocaine addiction may assist in the development of novel treatment strategies in the clinic.

The effect of prolonged blockade of DA uptake sites has been examined after chronic cocaine administration in order to determine if there are any long term changes in DA neurotransmission. According to Dackis and G[1]old (1985), there is indirect evidence to suggest that prolonged exposure to cocaine overstimulates DA neurons and leads to excessive synaptic metabolism and subsequent depletion of DA. An increase in DA D2 sites in the NAS (Goeders, 1990; Peris et al., 1990) shortly after drug withdrawal and evidence for D2 autoreceptor supersensitivity to pergolide in the striatum (Dwoskin et al., 1988) have been considered to indicate decreased levels of DA in rats. Decreased DA synthesis has also been reported following repeated exposure to cocaine (Brock et al., 1990).

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[1] A version of this chapter has been published: Burger and Martin-Iverson, 1993, *Synapse, 13:*20-29.

On the other hand, many studies do not support the notion of a cocaine-induced DA depletion (Henry et al., 1989; Ng et al., 1991; Pettit et al., 1990), and those designed to directly measure DA tissue levels in the brain have found no significant effects of chronic cocaine even with daily doses as high as 100 mg/kg (Kleven et al., 1988; Ryan et al., 1988). More recent research has employed *in vivo* microdialysis techniques to uncover decreased basal levels of extracellular DA in the striatum following a 10 day abstinence from cocaine (Parsons et al., 1991). This decrease in extracellular DA is indirectly supported by the finding of increased K^+-stimulated release of acetylcholine which, coupled with an increase in DA uptake sites after chronic cocaine, points to decreased synaptic levels of DA (Yi and Johnson, 1990). The conclusions drawn from these data indicate that cocaine exposure may result in decreased synaptic release of DA which would not necessarily be reflected in whole tissue levels. Additional measures are required in order to assess this alternate form of the DA depletion hypothesis.

With respect to the effect of cocaine on DA receptors, the general consensus is one of a decrease in the density of D2 sites in the striatum and an increase in D2 receptor sites in the NAS following 8 - 15 days of daily cocaine injections (Goeders, 1990; Kleven et al., 1990; Peris et.al, 1990). Kleven et al. (1990) also reported a significant decrease in striatal D1 receptor sites with this intermittent injection schedule. These data suggest an altered sensitivity of postsynaptic DA receptors, particularly striatal D1 receptors which remained decreased as long as 2 weeks after cocaine withdrawal (Kleven et al., 1990). However, an increase in behavioral sensitivity to the modulation of effects of a D1 agonist on effects of a D2 agonist has been associated with the behavioral sensitization that develops with psychomotor stimulant administration (Martin-Iverson et al., 1988a). Blockade of D1 receptors in the VTA blocks the behavioral sensitization to amphetamine (Stewart and Vezina, 1989). Furthermore, stimulation of D1 receptors has been shown to be important for the development of behavioral sensitization to apomorphine (Mattingly et al., 1991). Repeated cocaine administration increases the sensitivity of neurons to an exogenous D1, but not D2, receptor agonist (Henry and White, 1991). These D1 receptor effects in mediating behavioural sensitization do no fit with the reports of decreased D1 receptors in the striatum, suggesting that measurements of overall receptor density may not provide adequate information about the underlying changes with chronic cocaine treatment.

The changes in behavioral effects following prolonged exposure to stimulants like amphetamine and the D2 receptor agonist PHNO, for example, have been shown to be influenced by circadian rhythms (Martin-Iverson, 1991; Martin-Iverson and Iversen, 1989; Martin-Iverson et al., 1987; 1988a; 1988b; Martin-Iverson and Yamada, 1992). This work has revealed a pattern of daytime tolerance and nocturnal sensitization to the behavioral effects of these drugs when administered continuously *via* osmotic minipumps. The daytime tolerance can be reversed with administration of a D1 receptor agonist, and a D1 antagonist blocks the initial locomotor effects of a D2 agonist (Martin-Iverson et al., 1988a). Based on these data, it would seem that D1 receptor sensitivity is affected by chronic stimulant administration, with differential behavioral results depending on the time of day.

The purpose of the following study was to examine the effects of continuous cocaine infusion on DA receptor subtypes in the rat striatum. Day versus night differences in DA binding were investigated subsequent to an injection of EEDQ. This substance competitively denatures DA receptors but has a relatively moderate affinity for the receptor complex. Protection from EEDQ-induced reduction in the density of DA receptors therefore provides an indication of *in vivo* occupation of both D1 and D2 subtypes by endogenous and/or exogenous ligands. This method of receptor analysis was chosen to determine the effects of chronic cocaine on DA levels at specific binding sites, and, presumably at receptors.

3.2. MATERIALS AND METHODS

3.2.1. Animals

Male Sprague-Dawley rats (University of Alberta, Ellerslie) (n=192) weighing 250-350g were individually housed with food and water available ad libitum. The colony rooms were maintained at 22 °C. Half of the rats were kept on a normal 12 hour light-dark cycle (lights on at 0800 h), and the rest of the animals were kept on a reversed light-dark cycle (lights on at 2000 h) in order to ensure constancy in treatment duration at the time of sacrifice.

3.2.2. Drug Administration Procedure

After 10 days of habituation to the housing and lighting conditions, the rats were assigned to one of 16 drug groups in a counterbalanced fashion. Cocaine hydrochloride (0, 2.5, 7.5 or 22.5 mg/day) was administered either alone or in combination with the D1 antagonist, SCH 23390 (80 µg/day), the D2 antagonist, raclopride (4 mg/day), or both. All drugs were dissolved in double distilled water and delivered subcutaneously *via* Alzet osmotic minipumps (model 2ML2, Alza Corporation) designed to pump for over 15 days (n=12 per group). On Day 0, the rats were anesthetized with methoxyfluorane for pump implantation (between 1300-1600 hr). A subcutaneous pocket was formed through a small incision in the dorsal midscapular region of the skin. Upon insertion of the pump, the incision was closed with suture clips. Rats were given a single injection (5 mg/kg, i.p.) of EEDQ corresponding to either the light or dark portion of Day 15, depending on the colony lighting (n=6 per group). Therefore both lighting groups received the EEDQ injection at the same time relative to initiation of drug treatments. EEDQ was dissolved in ethanol, propylene glycol and distilled water (2:1:2). At 24 hours post-EEDQ injection, the rats were decapitated and their brains removed for dissection on ice (see Chapter 2). Both sides of the olfactory tubercle, NAS and striatum were collected and stored at -80 °C for neurochemical analysis. During the course of this experiment, rat tissue samples were decreased to n=4 or 5 in certain drug groups due to attrition or other procedural difficulties in tissue handling. All procedures involving animals received prior approval from the Health Sciences Animal Welfare Committee (HSAWC).

3.2.3. DA Receptor Binding

Unilateral striatal tissue from each rat (left and right sides randomized across assays) was subjected to radioligand binding assays for both D1 and D2 receptor labelling (see Chapter 2 for procedure).

3.2.4. Statistics

Receptor densities (Bmax) and affinities (K_d) were calculated from the data from the receptor binding assays using LIGAND and these values were subjected to ANOVA with four factors. These factors were: (1) cocaine treatment (COC) with four levels (vehicle, 2.5, 7.5, and 22.5 mg/day) (2) SCH 23390 treatment (SCH) consisting of two levels (vehicle or 80 μg/day) (3) raclopride treatment (RAC) with two levels (vehicle or 4 mg/day) and (4) portion of light-dark cycle when the rats were killed (LITDARK). Multiple F tests (Kiess, 1989) were applied for individual comparisons where appropriate.

3.3. RESULTS

3.3.1. D1 Receptor Binding

Chronic cocaine treatment produced a dose-dependent day *versus* night difference in D1 receptor protection from EEDQ in the rat striatum (Fig. 3-1). ANOVA on data from rats only receiving cocaine indicated a significant interaction between cocaine dose and day *versus* night ($F(3, 40)=3.56$, $p < .025$). In the 22.5 mg/day group, Bmax values were significantly greater in those rats decapitated during the day compared to both vehicle animals and those killed at night. In other words, during the light portion of the day-night cycle, cocaine treatment protected more D1 receptors from EEDQ inactivation (i.e. more D1 sites were occupied as a consequence of cocaine administration) than in the dark.

Administration of the D1 receptor antagonist SCH 23390 also resulted in a significant day *versus* night difference (Fig. 3-2). More D1 receptor protection was evident during the night compared to the day as revealed by a significant SCH x LITDARK interaction ($F(1,157) = 4.5$, $p<.05$). This finding was opposite to that seen with cocaine treatment and may explain why, when both SCH 23390 and cocaine were delivered simultaneously for 14

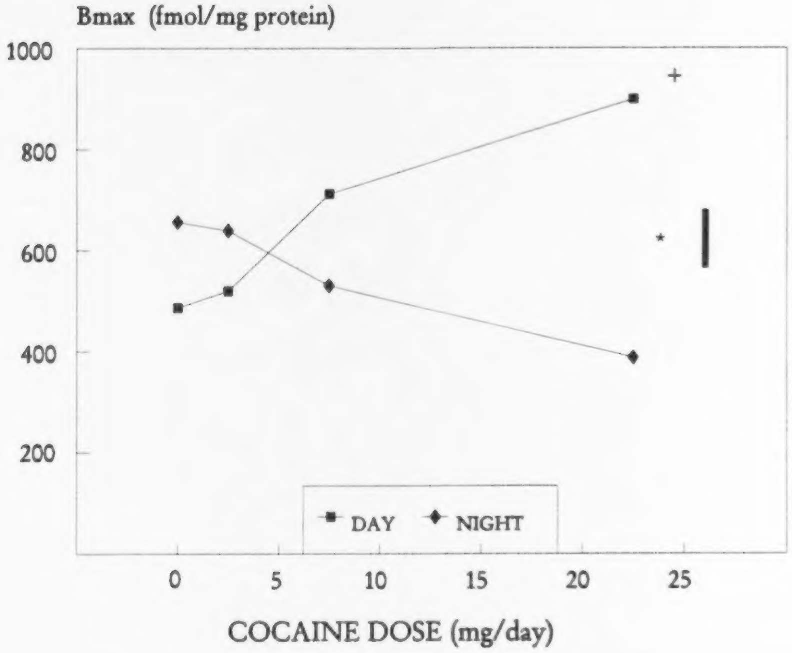

Fig. 3-1. D1 receptor densities measured as mean Bmax after treatment with EEDQ (5 mg/kg) and cocaine (0, 2.5, 7.5 and 22.5 mg/day). D1 receptor protection from EEDQ denaturation with increasing dose of cocaine revealed a significant day *versus* night difference for the 22.5 mg/day dose (*$p<.05$). Bmax in those rats decapitated during the day was increased dose-dependently (+$p<.05$, compared to vehicle controls) indicating greater protection from EEDQ than those killed at night. Line indicates SE of all means. n=6 per group.

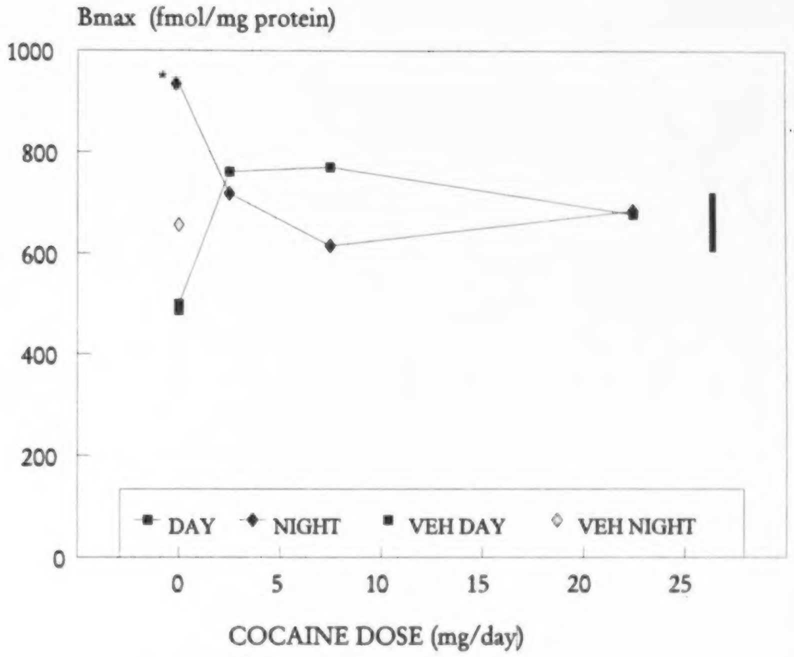

Fig. 3-2. Mean receptor densities (Bmax) after treatment with EEDQ (5 mg/kg) and cocaine (0, 2.5, 7.5 or 22.5 mg/day). Rats treated with SCH 23390 (80 μg/day) alone (0 mg/day cocaine) exhibited D1 receptor protection from EEDQ with significantly higher Bmax values at night compared to the day (*$p<.05$). Co-administration of SCH 23390 with increasing doses of cocaine served to eliminate any circadian differences. Line indicates SE of all means. n=5-6 per group.

days , no light-dark effect was apparent (Fig. 3-2). In these animals, *in vivo* occcupation of D1 receptor sites did not differ significantly from vehicle controls regardless of the time of day the tissue was taken.

Results of the 4 factor ANOVA for effects of cocaine, SCH 23390, raclopride and circadian cycle revealed a significant RAC effect (F(1,157) = 48.1, p<.001) and a RAC x COC x LITDARK interaction (F(3,157) = 3.46, p<.02) with respect to D1 receptor densities. Chronic raclopride treatment did not reveal a statistically significant day *versus* night difference but lower Bmax values were found at night compared to vehicle control (Fig. 3-3). The addition of cocaine did not significantly affect D1 receptor protection from EEDQ compared to raclopride alone. Multiple F tests did, however, reveal a significant decrease in Bmax in those groups receiving raclopride and cocaine compared to cocaine alone (compare Fig. 3-1 and Fig. 3-3). More specifically, raclopride administered in conjunction with the 2.5 mg/day dose of cocaine significantly decreased D1 receptor protection during the night compared to this low dose of cocaine alone. This was also found during the day but for the RAC+COC7.5 and RAC+COC22.5 mg/day drug combinations (Fig. 3-3). Raclopride, therefore, decreased D1 receptor protection from EEDQ both alone and in combination with cocaine compared to vehicle and cocaine alone controls respectively, with differential effects depending on the light-dark cycle.

Interestingly, administration of both receptor antagonists (SCH+RAC) in a chronic fashion did not significantly alter Bmax values for the D1 receptor (Fig. 3-4). In those animals who also received cocaine for 14 days, no significant differences were reported compared to vehicle control. The SCH+RAC+COC22.5 group did show a signficant

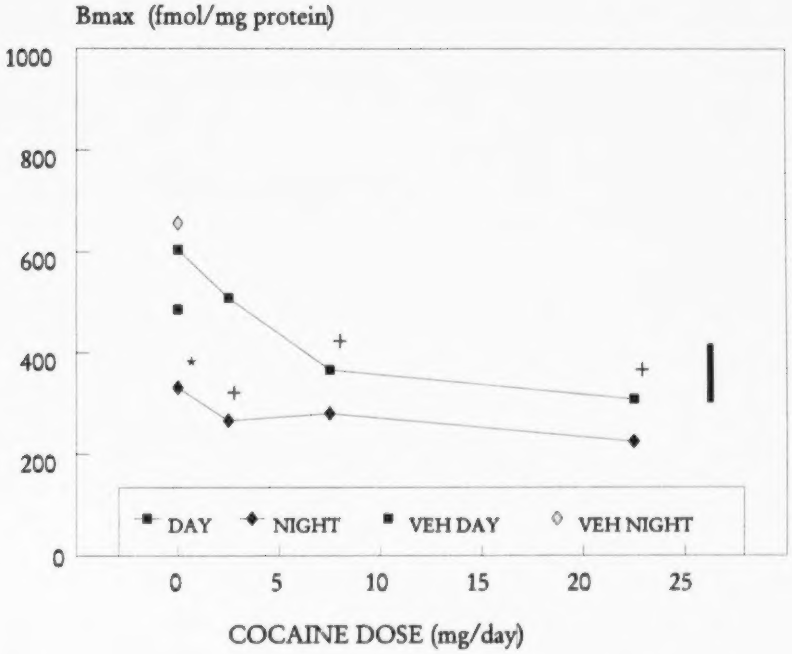

Fig. 3-3. Mean D1 receptor densities (Bmax) after treatment with EEDQ (5 mg/kg) and cocaine (0, 2.5, 7.5 or 22.5 mg/day). The D2 antagonist raclopride (4 mg/day) significantly decreased D1 receptor protection from EEDQ at night compared to vehicle controls (*p<.05). Co-administration of raclopride with cocaine also resulted in significant decreases in D1 receptor Bmax compared to cocaine controls (+p<.05). Line indicates SE of all means. n=6 per group.

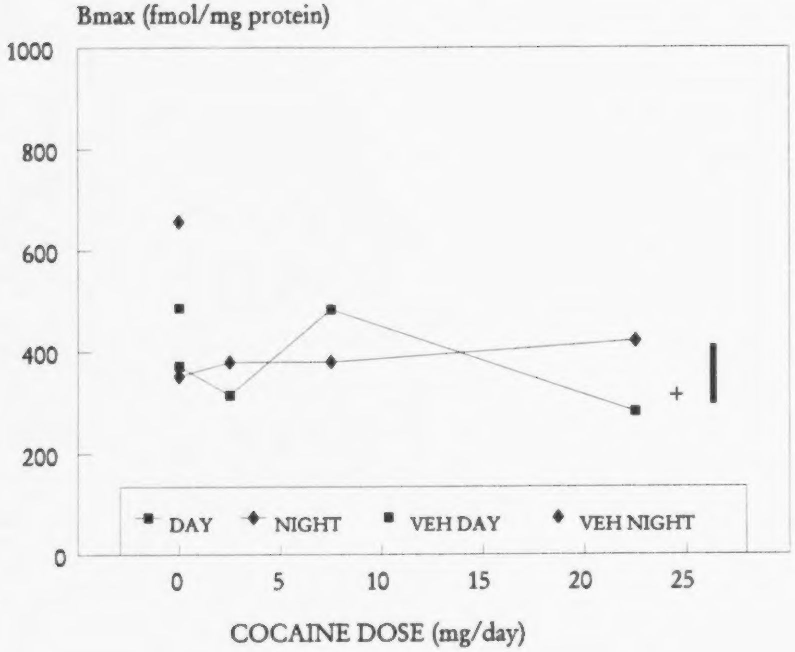

Fig. 3-4. Mean Bmax values of D1 receptors in rats treated with SCH23390 (80 μg/day) and raclopride (4 mg/day) and one of 4 doses of cocaine determined from animals killed during the day or night. No significant changes in D1 receptor protection from EEDQ denaturation were found in rats receiving chronic treatment of SCH 23390 and raclopride together. In rats receiving both antagonists as well as cocaine, the SCH+RAC+COC22.5 group was found to have fewer protected D1 receptors at night compared to COC22.5 controls (+$p<.05$). Line indicates SE of all means. n=5-6 per group.

decrease in *in vivo* D1 receptor occupation compared to the COC22.5 rats, but only during the day (Fig. 3-4). This finding resembles that seen in the RAC+COC22.5 group and so may be largely due to the D2 antagonist effect.

In contrast to the effects on Bmax, chronic cocaine did not significantly affect the affinity (K_d) of [^{3}H]SCH 23390 for D1 receptor binding sites in the striatum in either the light or dark portion of the day/night cycle (Table 3-1). However, an overall RAC effect was found (F(1,157) =21.82, p<.001) indicating receptor affinity changes with chronic antagonist treatment.

3.3.2. D2 Receptor Binding

The major finding regarding D2 receptor affects with chronic cocaine treatment was an overall effect of raclopride treatment (F(1,191) = 169.86, p<.001). As illustrated in Figure 3-5, those animals receiving chronic raclopride treatment either alone or in combination with cocaine and/or SCH 23390 had greater D2 receptor protection from EEDQ denaturation than those groups without raclopride. In addition, unlike D1 receptor Bmax effects, no dose-dependent day *versus* night differences were reported with cocaine treatment. A significant day/night effect was found in the 2.5 mg/day cocaine dose group (day=172.6 ± 64.1; night = 69.0 ± 14.7 fmol/mg protein, mean ± SEM) but no LITDARK effects were reported with ANOVA.

Analysis of K_d values with [^{3}H] spiperone binding to D2 receptor sites did not reveal any significant cocaine effects but, as with the D1 results, an overall RAC effect was noted (F(1,154) = 23.45, p<.001)(Table 3-1).

Table 3-1. Average K_d values (means ± SEM; expressed in nM) in rat striatum with chronic infusion of cocaine (COC:at doses shown, mg/day) and/or DA receptor antagonists (SCH=SCH23390, 80 μg/day; RAC=raclopride, 4 mg/day) and a single treatment with EEDQ (5 mg/kg). *Significantly different from vehicle control, p<.05. **Signficantly different from cocaine control, p<.05. ***Significantly different from without raclopride, p<.05.

Drug Group	^{3}[H]SCH 23390		^{3}[H] Spiperone	
	Light	Dark	Light	Dark
Vehicle	0.328 ± .06	0.281 ± .05	0.05 ± .01	0.07 ± .02
COC2.5	0.354 .13	0.795 .39	0.064 .01	0.063 .02
COC7.5	0.226 .02	0.342 .06	0.051 .02	0.073 .01
COC22.5	0.462 .21	0.343 .10	0.133* .09	0.044 .01
SCH	0.315 .05	0.435 .10	0.063 .02	0.051 .01
SCH+COC2.5	0.440 .08	0.354 .08	0.056 .02	0.039 .01
SCH+COC7.5	0.449 .10	0.289 .03	0.052 .01	0.055 .01
SCH+COC22.5	0.496 .08	0.348 .05	0.058 .01	0.048 .01
RAC	0.172 .01	0.218 .02	0.032 .01	0.029 .01
RAC+COC2.5	0.311 .10	0.179** .02	0.034 .01	0.020 .002
RAC+COC7.5	0.182 .03	0.234 .08	0.032 .01	0.029 .01
RAC+COC22.5	0.191 .02	0.215 .04	0.021 .001	0.033 .01
SCH+RAC	0.264 .05	0.187 .02	0.028 .01	0.021 .001
SCH+RAC+COC2.5	0.184 .01	0.244** .04	0.022 .001	0.024 .004
SCH+RAC+COC7.5	0.255 .01	0.212 .02	0.020 .002	0.018 .001
SCH+RAC+COC22.5	0.193 .01	0.359 .11	0.019 .002	0.048 .02
With RAC	0.218*** .02	0.231*** .02	0.026*** .01	0.028***.003
Without RAC	0.384 .04	0.401 .06	0.066	0.056 .004

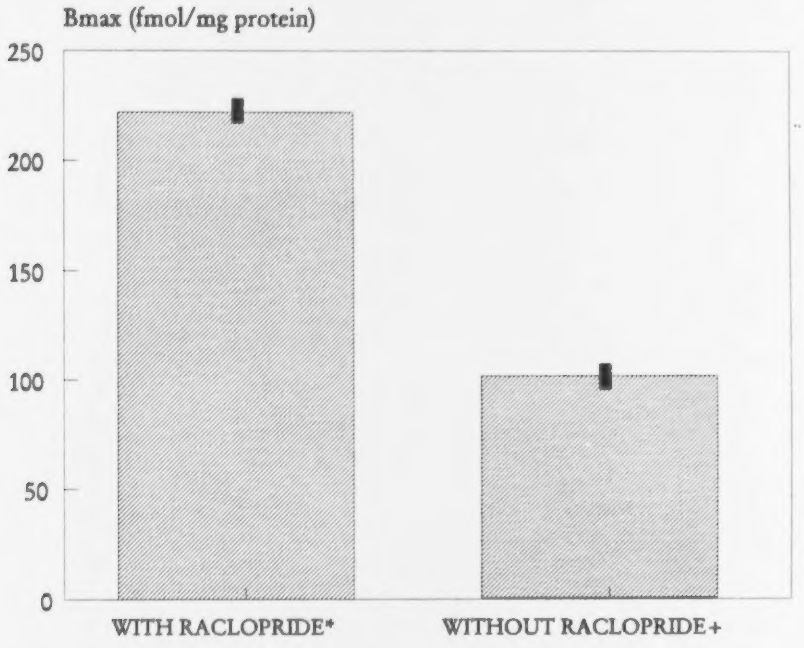

Fig. 3-5. D2 receptor density as mean Bmax ±SEM values in rats treated with or without raclopride (4 mg/day). D2 receptor protection from EEDQ was significantly higher in those rats receiving raclopride for 14 days prior to denaturation ($p<.001$). No circadian effects were found in D2 receptor density in any drug groups following EEDQ administration. *n=93. +n=94.

3.4. DISCUSSION

The present data indicate that cocaine administered continuously for 2 weeks produces a dose-dependent increase in striatal DA D1 receptor protection from EEDQ during the day, but a decrease in this protection at night. D2 receptor protection from EEDQ is not affected by the light/dark cycle or by chronic cocaine treatment.

Acute cocaine treatment increases extracellular DA levels *via* blockade of DA uptake (Ritz et al., 1987), and protects both D1 and D2 receptors from EEDQ-denaturation (Guhle et al., 1990). EEDQ therefore enables measurement of synaptic levels of DA by assessing DA receptor density after unoccupied receptor sites have been denatured. The results of the present experiment indicate that chronic treatment of rats with cocaine has differential effects on synaptic DA levels depending on the light/dark cycle, and is selective for those synapses which contain D1 receptors.

The lack of effects of chronic cocaine treatment on the *in vivo* occupation of D2 receptors is likely due to homeostatic mechanisms that compensate over time for the initial increases in extracellular DA induced by cocaine. These mechanisms include an increase in the density of DA uptake sites (Yi and Johnson, 1990) and reduced D2 receptor densities in striatum (Goeders, 1990; Kleven et al., 1990; Peris et.al, 1990). This result adds to the accumulating evidence that points to D2 receptors being involved in tonic dopaminergic activity (Martin-Iverson et al., 1987, 1988a, 1988b; Martin-Iverson 1991), such that perturbations of the D2 "system" are closely regulated by a variety of feedback mechanisms to ensure homeostatic functioning.

During the day, chronic cocaine apparently increases the extracellular concentration of DA at synapses containing D1 receptors. This effect is exemplified by the increases in D1 receptor Bmax in the presence of EEDQ with increasing doses of cocaine. At night, cocaine appears to reduce DA binding at the D1 receptor relative to Bmax values reported during the day. These observations raise the question as to how continuous administration of a constant dose of cocaine can produce opposite effects on the occupation of D1 receptors by DA in the day and night. Previous research has indicated that constant infusions of a D2 agonist can result in opposing effects depending on the day/night cycle; that is, behavioral tolerance during the day and sensitization at night (Martin-Iverson et al., 1988b). In the present case, however, we have evidence of an increase in DA synaptic levels during the day and a decrease at night. As mentioned previously, it has been reported that chronic cocaine treatment results in increased densities of DA uptake sites (Yi and Johnson, 1990). It is possible, although not yet tested, that there are circadian rhythms in densities of uptake sites, such that chronic cocaine produces supersensitive uptake sites during the night but not during the day. A more plausible mechanism might involve the circadian differences found in DA turnover whereby turnover is lower during the day compared to night (Friedman and Walker, 1968; Lemmer and Berger, 1978; O'Neil and Fillenz, 1985). It is of interest in this regard that we observed a trend towards higher densities of occupied D1 receptors in the striata of vehicle animals during the night as compared to during the day. With chronic cocaine administration, uptake inhibition may have a more marked effect when DA turnover is low (i.e. during the day). This possibility is supported by the finding that the effect of DA uptake blockers is dependent upon the electrical activity of the DA cell (Stamford et al., 1987). It has been suggested by Wood and Altar (1988) that DA uptake is a significant process for the inactivation of DA only under conditions of relatively low levels of cellular activity.

Another possible explanation for these findings is that negative feedback mechanisms may be more effective at night, under higher basal levels of dopaminergic activity, than during the day. Feedback mechanisms decreasing DA release include terminal D2 autoreceptors in the striatum (DiChiara et al., 1977; Kehr et al., 1972), both D1 and D2 somatodendritic autoreceptors in the SN (Argiolas et al. 1982; Savasta et al., 1986), and a long-loop striato-nigral GABAergic projection (Carlsson and Lindquist, 1963; Gerfen and Young, 1988; Nagy and Fibiger, 1980), activities of which may alter with day and night, and which may be responsible for the decreased nocturnal occupation of D1 receptors after chronic cocaine. It is clear that cocaine does inhibit the electrical activity of DA neurons, and evidence has been presented from in vitro work with SN slices indicating a role for somatodendritic autoreceptors (Lacey et al., 1990). However, whether or not there could be day/night differences in these feedback mechanisms have not been demonstrated beyond the differences in DA turnover itself.

It is also feasible that the difference between day and night in D1 receptor occupation is related to DA receptor turnover. There is evidence to suggest that D1 receptors have a fast turnover rate relative to D2 receptors in various brain regions (Fuxe et al., 1987). Indeed, the reported rate of production of D1 receptors (16.47 fmol/mg/h) would result in the production of a 200 fmol difference over 12 hours, which is close to the difference that we have found between day and night. That is, the day/night difference in D1 receptor densities could be explained on the basis of circadian differences in D1 receptor turnover, including the production, degradation and/or internalization of these specific DA receptors, as occurs with ß-adrenergic receptors (Levitzki, 1988).

Another question raised by the present results is how can continuous systemic cocaine administration differentially affect DA levels at D1 and D2 receptors? It seems likely that anatomical differences in the distributions of striatal D1 and D2 receptors within the striatum and of inputs to these synapses from dopaminergic or other neurons would be necessary for the observed results. Evidence exists to suggest that DA receptors are represented differentially in the rat striatum with D1 binding sites preferentially distributed in the striosomes (Besson et al., 1988) and higher relative densities of D2 receptors in the matrix (Loopuijt et al., 1987). Furthermore, distinct subdivisions of the A8-A9-A10 DA-containing neurons project preferentially to either the striosomal (patches) or matrix portions of the striatum (Jimenez-Castellanos and Graybiel, 1987). This differentiation in DA receptor distribution and inputs from different DA releasing neurons may help explain why only D1 receptors in the rat striatum are subject to phasic fluctuations in occupancy, providing that the anatomical differences are related to functional differences. We suggest the possibility that DA neurons projecting preferentially to striosomes primarily subserve a phasic function, while those that terminate to a greater extent in the striatal matrix are more involved in tonic regulation.

The circadian effects reported with respect to D1, but not D2, receptor occupancy are also exemplified in the rats given chronic treatment with the D1 antagonist, SCH 23390. The SCH 23390 group revealed a significant day *versus* night effect in D1 receptor Bmax that was opposite in direction to cocaine's effects on the D1 receptor. Since a constant dose of SCH is administered, greater protection of D1 receptors at night than during the day in this drug group implies that there is a circadian rhythm in DA release or in D1 receptor densities. That is, greater protection occurs at night due to higher nocturnal DA release or higher nocturnal D1 receptor density. It is notable that vehicle-treated animals exhibit a trend in this direction. When both the antagonist and cocaine were administered simultaneously, no circadian differences were apparent. SCH 23390 treatment did not have any significant effect on the D2 receptor.

Raclopride treatment, on the other hand, not only protected D2 sites from EEDQ denaturation, as expected, but also had an effect on D1 receptor occupancy. Rats receiving raclopride alone or in combination with cocaine revealed decreased D1 receptor Bmax values compared to vehicle and cocaine controls, respectively. These findings may be explained by the fact that raclopride would selectively protect D2 sites from EEDQ, thereby increasing the concentration of EEDQ at D1 sites vulnerable to inactivation. Alternatively, chronic raclopride may induce supersensitivity of autoreceptors regulating DA release such that less DA is released at D1 sites. The finding of main effects on receptor affinity by raclopride treatment for both D1 and D2 receptors suggests, however, that chronic D2 antagonist treatment somehow affects the binding of the DA receptor *in vitro*. Since the K_d value was decreased by raclopride, the possibility of the drug still being present in the tissue at the time of death producing these effects can be ruled out, as this scenerio would cause an increase in K_d. Decreased K_d values suggest that the Bmax data do not accurately reflect receptor changes *in vivo* since the measurement of receptor density *in vitro* is dependent on the affinity of the receptor for the radioligand. In essence, the effects of raclopride on D1 receptor occupancy are questionable in this case. That this drug administration procedure affects DA receptor affinity is clearly apparent in that it is observed with large group sizes (in this case, n=93).

Based on the data presented in this study, it can be concluded that cocaine's effects are influenced by circadian rhythms in the DA system. More specifically, the D1 receptor may be said to act in a phasic manner in response to the day/night differences in DA function. Circadian effects and D1 receptor activity have previously been associated in the report of a reversal of daytime behavioral tolerance to PHNO, a direct D2 agonist, with administration of a D1 agonist (Martin-Iverson et al., 1988a). In addition, the D1 antagonist SCH 23390 was found to attentuate the initial nocturnal stimulant effects of PHNO. These data, taken with the present findings, implicate the D1 receptor in the circadian rhythm effects on stimulant drug activity. It has previously been reported that D1 agonists but not D2 agonists affect the period of the circadian rhythms of rats maintained under constant lighting conditions (Yamada and Martin-Iverson, 1991). Furthermore, there is a relatively high density of D1 receptors (but not D2 receptors) in the suprachiasmatic nucleus, the "pacemaker" of mammalian circadian rhythms (Boyson et al., 1986). It appears that not only is there a D1 role in regulating circadian rhythms, but circadian rhythms selectively influence the occupation of D1 receptors by DA.

The phasic role of the D1 receptor is important to the hypothesis that psychoses may arise from transient D1 activation in the face of tonic D2 stimulation. It has been proposed that behaviorally relevant stimuli induce phasic DA release while tonic DA release, regulated by glutamatergic projections, serves to set the background level of DA in the extracellular space (Grace, 1991). The tonic DA release is homeostatically controlled and most likely involves the D2 receptor since it mediates autoreceptor inhibition of DA synthesis and release. Phasic DA release is transient and can be induced by stress, pharmacological intervention (i.e. psychostimulant administration) and changes in the circadian cycle. We suggest that these effects are mediated by D1 receptor activation. In the case of schizophrenia, the background or tonic DA D2 activation is altered by the disease process such that phasic D1 activation would instigate an exaggerated response manifest as a psychotic episode. Such a case occurs with continuous systemic infusions of a selective D2 receptor agonist. Tolerance develops to the initial effects of the drug during the day. However, an acute stressor presented during tolerance results in a phasic exaggerated response (Martin-Iverson et al., 1988b). This phasic exaggerated response to stress is blocked by administration of a D1 antagonist, and mimicked by a D1 agonist (Martin-Iverson et al., 1988a). This may explain why stress and psychostimulant drugs exacerbate the positive symptoms of schizophrenia, psychotic episodes which are only periodically expressed, often as a result of a stressful experience (Angrist, 1983). The phasic D1, tonic D2 receptor hypothesis also finds support in the aforementioned finding that D1 receptors have a faster turnover rate in the striatum, nucleus accumbens and olfactory tubercle compared to D2 receptors (Fuxe et al., 1987). The suggestion that the D1 receptor may require dynamic regulation in order to respond to changes in demand for D1 receptor activity implicates a transient or phasic role.

The present findings have implications for the DA depletion hypothesis proposed by Dackis and Gold (1985). Although indirect measures of dopaminergic activity have suggested that chronic cocaine depletes DA levels in the brain, direct assessment of DA tissue levels have failed to confirm these reports (Kleven et al., 1988). Additional research has reported changes in extracellular DA, leading to the suggestion that cocaine treatment causes decreased synaptic release of DA. The data in the present experiment support the notion that cocaine alters the levels of DA selectively at sites containing D1 receptors, and that both increases and decreases in the amount of DA interacting with D1 receptors occur as a function of day and night, respectively. Furthermore, the present data indicate that changes in the D2 receptor system after chronic cocaine compensate for the acute effects of cocaine. The *in vivo* occupation of D2 receptors is the same for vehicle- and cocaine-treated rats after 14 days of treatment.

In conclusion, the results indicate that: 1) cocaine protected D1 receptors from EEDQ in a dose-dependent fashion during the day, but decreased protection of D1 receptors at night; 2) since these effects of cocaine reflect day/night differences in cocaine's actions on synaptic levels of DA, D1 receptor occupation *in vivo* is likely increased during the day and decreased at night with chronic cocaine administration; 3) co-administration of DA receptor antagonists with cocaine eliminated the day *versus* night difference and, in the case of the D1 antagonist SCH 23390, produced the opposite effect on D1 receptor occupation when administered alone; 4) occupation of D1 receptors is phasic in nature and is influenced by circadian changes in the DA system; 5) the D2 receptor "system" can compensate for cocaine's effects on the *in vivo* occupation of the D2 receptors with chronic treatment; 6) chronic treatment with raclopride alters the affinities of D1 receptors to [^{3}H] SCH 23390 and of D2 receptors to [^{3}H] spiperone. In general, these data add to the growing evidence that D1 receptors are involved in more phasic dopaminergic events, while D2 receptors may be involved in tonic processes.

3.5. REFERENCES

Angrist, B. (1983) Psychoses induced by central nervous system stimulants and related drugs, In Creese, I. (Ed), *Stimulants: Neurochemical, behavioural, and clinical perspectives*, Raven Press, New York, pp. 1-30.

Argiolas, A, Melis, M.R., Fadda, F. and Gessa, G.L. (1982) Evidence for dopamine autoreceptors controlling dopamine synthesis in the substantia nigra. *Brain Res.*, *234*:177-181.

Besson, M-J., Graybiel, A.M. and Nastuk, M.A. (1988) [^{3}H]SCH 23390 binding to D1 dopamine receptors in the basal ganglia of the cat and primate: delineation of striosomal compartments and pallidal and nigral subdivisions. *Neurosci.*, *26:*101-119.

Boyson, S.J., McGonigle, P. and Molinoff, B. (1986) Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in the brain. *J. Neurosci.*, *6*:3177-3188.

Brock, J.W., Ng, J.P., and Justice, J.B. Jr. (1990) Effect of chronic cocaine on dopamine synthesis in the nucleus accumbens as determined by microdialysis perfusion with NSD-1015. *Neurosci. Lett.*, *117*:234-239.

Carlsson, A. and Lindquist, M. (1963) Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacol. Toxicol. (Copenh.)*, *20:*140.

Dackis, C.A. and Gold, M.S. (1985) New concepts in cocaine addiction: The dopamine depletion hypothesis. *Neurosci. Biobehav. Rev.*, *9:*469-477.

DiChiara, G., Porceddu, M.L., Spano, P.F. and Gessa, G.L. (1977) Haloperidol increases and apomorphine decreases striatal dopamine metabolism after destruction of striatal dopamine-sensitive adenylate cyclase by kainic acid. *Brain Res., 130*:374-382.

Dwoskin, L.P, Peris, J., Yasuda, R., Philpott, K. and Zahniser, N.R. (1988) Repeated cocaine administration results in supersensitivity of striatal D2 dopamine autoreceptors to pergolide. *Life Sci., 42:*255-262.

Friedman, A.H. and Walker, C.A. (1968) Circadian rhythms in rat mid-brain and caudate nucleus biogenic amine levels. *J. Physiol. (London), 197*:77-85.

Fuxe, K., Agnati, L.F., Pich, E.M., Meller, E., and Goldstein, M. (1987) Evidence for fast receptor turnover of D1 dopamine receptors in various forebrain regions of the rat. *Neurosci. Lett., 81:*183-187.

Gerfen, C.R. and Young, W.S. (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both paatch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Res., 460*:161-167.

Goeders, N.E. (1990). The effects of chronic cocaine administration on brain neurotransmitter receptors. *Drug Devel. Res., 20:*349-357.

Grace, A.A. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neurosci., 41*:1-24.

Guhle, M.E., Singh, S. and Martin-Iverson, M.T. (1990) The effect of in vivo N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline on rat striatal dopamine receptor densities. *Heritage Medical Research Days Abstracts, #46.*

Henry, D.J., Greene, M.A., and White, F.J. (1989) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Repeated administration. *J. Pharmacol. Exp. Ther., 251:*833-839.

Henry, D.J. and White, F.J. (1991) Repeated cocaine administration causes persistent enhancement of D1 dopamine receptor supersensitivity within the rat nucleus accumbens. *J. Pharmacol. Exper. Ther., 258*:882-890.

Hurd, Y.L. and Ungerstedt, U. (1989) Cocaine: an in vivo microdialysis evaluation of its acute action on dopamine transmission in rat striatum. *Synapse, 3*:48-54.

Jimenez-Castellanos, J. and Graybiel, A.M. (1987) Subdivisions of the dopamine-containing A8-A9-A10 complex identified by their differential mesostriatal innervation of striosomes and extrastriosomal matrix. *Neurosci., 23*:223-242.

Kalivas, P.W. and Duffy, P. (1990) Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse, 5:*48-58.

Kehr, W., Carlsson, A., Lindqvist, M., Magnusson, T. and Atack, C. (1972) Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity. *J. Pharmacol., 24:*744-747.

Kiess, H.O. (1989) Statistical Concepts for the Behavioral Sciences. Allyn and Bacon, Boston, pp. 326-331.

Kleven, M.S., Perry, B.D., Woolverton, W.L. and Seiden, L.S. (1990) Effects of repeated injections of cocaine on D1 and D2 dopamine receptors in rat brain. *Brain Res., 532:*265-270.

Kleven, M.S., Woolverton, W.L. and Seiden, L.S. (1988) Lack of long-term monoamine depletions following repeated or continuous exposure to cocaine.*Brain Res. Bull., 21*:233-237.

Kuhar, M.J., Ritz, M.C. and Boja, J.W. (1991) The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci., 14*:299-302.

Lacey, M.G., Mercuri, N.B. and North, R.A. (1990) Actions of cocaine on rat dopaminergic neurones *in vitro*. *Br. J. Pharmacol., 99*:731-735.

Lemmer, B. and Berger, T. (1978) Diurnal rhythm in the central dopamine turnover in the rat. *Naunyn-Schmiedeberg's Arch. Pharmacol., 303*:257-261.

Levitzki, A. (1988) From epinephrine to cyclic AMP. *Science, 241*:800-806.

Loopuijt, L.D., Sebens, J.B. and Korf, J. (1987) A mosaic-like distribution of dopamine receptors in rat neostriatum and its relationship to striosomes. *Brain Res., 405*:405-408.

Martin-Iverson, M.T. (1991) An animal model of stimulant-induced psychoses. In:A.A. Boulton, G.B. Baker, M.T. Martin-Iverson, eds. *Animal models in psychiatry I. Vol. 18.* Humana Press, Totowa, NJ, pp. 103-149.

Martin-Iverson, M.T. and Iversen, S.D. (1989) Day and night locomotor activity effects during administration of (+)-amphetamine. *Pharmacol. Biochem. Behav., 34:* 465-471.

Martin-Iverson, M.T., Iversen, S.D. and Stahl, S.M. (1988a) Long-term motor stimulant effects of (+)-4-propyl-9-hydroxynaphthoxazine (PHNO), a dopamine D2 receptor agonist: interactions with a dopamine D1 receptor antagonist and agonist. *Eur. J. Pharmacol., 149*:25-31.

Martin-Iverson, M.T., Stahl, S.M. and Iversen, S.D. (1987) Factors determining the behavioural consequences of continuous treatment with 4-propyl-9-hydroxynaphthoxazine, a selective dopamine D2 agonist. In: *Parkinson's Disease: Clinical and experimental advances.* F.C. Rose, ed. J. Libbey and Co., London, pp.169-177.

Martin-Iverson, M.T., Stahl, S.M. and Iversen, S.D. (1988b) Chronic administration of a selective dopamine D2 agonist: factors determining behavioral tolerance and sensitization. *Psychopharmacol., 95*:534-539.

Martin-Iverson, M.T. and Yamada, N. (1992) Synergistic behavioral effects of dopamine D1 and D2 agonists are determined by circadian rhythms. *Eur. J. Pharmacol., 215:*119-125.

Mattingly, B.A., Rowlett, J.K., Graff, J.T. and Hatton, B.J. (1991) Effects of selective D1 and D2 dopamine antagonists on the development of behavioral sensitization to apomorphine. *Psychopharmacol., 105*:501-507.

Nagy, J.I. and Fibiger, H.C. (1980) A striatal source of glutamic acid decarboxylase activity in the substantia nigra. *Brain Res., 187:*237-242.

Ng, J.P., Hubert, G.W., and Justice, J.B. Jr. (1991) Increased stimulated release and uptake of dopamine in nucleus accumbens after repeated cocaine administration as measured by in vivo voltammetry. *J. Neurochem., 56:*1485-1492.

O'Neill, R.D. and Fillenz, M. (1985) Simultaneous monitoring of dopamine release in rat frontal cortex, nucleus accumbens and striatum: effect of drugs, circadian changes and correlations with motor activity. *Neurosci., 16*:49-55.

Parsons, L.H., Smith, A.D., and Justice, J.B. Jr. (1991) Basal extracellular dopamine is decreased in the rat nucleus accumbens during abstinence from chronic cocaine. *Synapse, 9:*60-65.

Peris, J., Boyson, S.J., Cass, W.A., Curella, P., Dwoskin, L.P., Larson, G. Lin, L., Yasuda, R.P. and Zahniser, N.R. (1990) Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. *J. Pharmacol. Exp. Ther., 253*:38-44.

Pettit, H.O., Pan, H.-T., Parson, L.H. and Justice, J.B. Jr. (1990) Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. *J. Neurochem., 55:*798-804.

Ritz, M.C., Lamb, R.J., Goldberg, S.R. and Kuhar, M.J. (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 237:*1219-1223.

Ryan, L.J., Martone, M.E., Linder, J.C., and Groves, P.M. (1988) Cocaine, in contrast to d-amphetamine, does not cause axonal terminal degeneration in neostriatum and agranular frontal cortex of Long-Evans rats. *Life Sci., 43:* 1403-1409.

Savasta, M., Dubois, A., Benavides, J. and Scatton, B. (1986) Different neuronal location of [^{3}H]SCH 23390 binding sites in pars reticulata and pars compacta of the substantia nigra in the rat. *Neurosci. Lett., 72*:265-271.

Stamford, J.A., Kruk, Z.L. and Millar, J. (1987) Accommodation of rat nigrostriatal dopamine neurones to high frequency electrical stimulation of the medial forebrain bundle: *in vivo* voltammetric data. *Neurosci. Lett., 82:*172-176.

Stewart,J. and Vezina, P. (1989) Microinjections of SCH 23390 into the ventral tegmental area and substantia nigra pars reticulata attenuate the development of sensitization to the locomotor activating effects of systemic amphetamine. *Brain Res., 495:*401-406.

Wood, P.L. and Altar, C.A. (1988) Dopamine release *in vivo* from nigrostriatal, mesolimbic, and mesocortical neurons: utility of 3-methoxytyramine measurements. *Pharmacol. Rev., 40:*163-187.

Yamada, N. and Martin-Iverson, M.T. (1991) Selective dopamine D1 and D2 agonists independently affect different components of the free-running rhythm of locomotor activity in rats. *Brain Res., 538:*310-312.

Yi, S.J. and Johnson, K.M. (1990) Effects of acute and chronic administration of cocaine on striatal uptake, compartmentalization and release of [^{3}H]dopamine. *Neuropharmacol., 29:*475-486.

CHAPTER 4. THE EFFECTS OF CONTINUOUS COCAINE ADMINISTRATION ON LOCOMOTION AND THE STRUCTURE OF BEHAVIOR

4.1. INTRODUCTION

Previous work with psychomotor stimulants has shown that regimens of administration, dose and day/night cycle influence the behavioral effects of these drugs when administered chronically. Daily intermittent injections produced sensitization of locomotion and other behaviours such as sniffing, rearing and head movements (Angrist, 1983; Segal and Shuckit, 1983). This sensitization varies in intensity and duration depending on dose and number of injections. With cocaine, for example, a single dose of 40 mg/kg can produce sensitization that lasts approximately one week while 10 days of a 10 mg/kg dose produces sensitization that endures for months (Post et al., 1992). When sensitization-inducing doses are administered continuously by subcutaneously implanted pumps or pellets, however, behavioural tolerance results. This opposite effect on behaviour is a function of both the route and regimen of administration but may also depend on the day/night cycle. With amphetamine (Martin-Iverson and Iversen, 1989) and the D2 agonist (+)-4-propyl-9-hydroxynaphthoxazine (PHNO) (Martin-Iverson et al., 1988a), continuous infusion results in tolerance during the daytime hours only. At night, the animals exhibit sensitization to the motor stimulant effects of these drugs. These results underscore the importance of the animal's circadian cycle in interpreting the effects of chronic psychomotor stimulants.

Analysis of the behavioural effects of stimulant drugs has ranged from general activity assessments like locomotor activity to intensity ratings designed to assess the degree of stereotypy induced by these drugs. Several theories of behavioural stimulant effects have emerged regarding the structures of these responses to psychomotor stimulant administration. Damianopoulos and Carey (1992) report that behavioural sensitization to cocaine occurs only relative to control animals that habituated to the drug environment. Close analysis of the structure of this locomotor behaviour, however, demonstrates significant increases in the diameter of the turns made in the test box, indicating a pattern of behaviour that has widened to include more features of the environment. According to these authors, the behavioural result of repeated stimulant administration is a function of the environmental context and involves a restructuring of behaviour that does not necessarily coincide with locomotor sensitization. This behavioural reorganization process depends, however, on the specific environment and the animal's behavioural response to the cues therein (Damianopoulos and Carey, 1993). In this framework, hyperlocomotion is not the required outcome of repeated drug administration since locomotor activity may not become part of the response to environmental stimuli experienced in the drug state. This is evidenced by the finding that some rats remain in a state of hypolocomotion as they engage in repetitive sniffing and licking of proximal environmental features (Damianopoulos and Carey, 1993). Other theories on the behavioural effects of psychomotor stimulant treatment have also focused on the underlying structural changes after repeated exposure. The Lyons and Robbins (1975) hypothesis, for example, proposes an inverse relationship between amphetamine dose and the number of response categories. As the dose of amphetamine increases, certain behaviours are selectively increased at the expense of others, due to behavioural competition. This process involves a shortening of response durations for some behaviours, increased transitions between response sequences, shortened or abortive response sequences and increases in the rate of some behaviours when others are reduced (Robbins et al., 1990). The stereotypy-inducing properties of amphetamine have thus been analyzed beyond simple ratings of intensity to include assessments of a number of behaviours and the sequences between them.

Within this theoretical framework, a dose-response analysis of cocaine's effects on behaviour is of interest, given that previous reports have been less than complete. Research on the behavioural effects of continuous cocaine has concluded that subjects become tolerant to the stimulant effects after chronic exposure (Reith et al., 1987; King et al., 1992). This finding is reported in response to a challenge dose after chronic treatment for measures of both locomotor activity and/or stereotypy rating scores. Reith et al. (1987) also measured behaviour throughout the continuous administration phase of cocaine treatment and reported locomotor stimulation for the first 11 days and 7 nights, followed by tolerance in the last week of drug delivery. Due to the high drug doses (25 - 150 mg/kg/day) used in this study, however, only the lowest drug group survived for full behavioural analysis. Furthermore, a complete analysis of the structure of behaviour during this time period was not provided. Given the stereotypy-inducing properties of this drug, it is important to examine changes in the structure of cocaine-induced behaviour throughout the chronic treatment period. Therefore, it is of interest to assess both general and specific behavioural activities in response to several doses of cocaine administered continuously over both the light and dark portions of the circadian cycle.

The present experiment examines the effect of cocaine on 28 separate behaviours recorded and coded from both the first and 14th 24 hour period of drug treatment. This behavioural analysis included frequency and duration of bouts and transitions between behaviours as a measure of the temporal organization of behaviour. In addition, locomotor activity was measured hourly throughout the two-week period. This study therefore provides extensive information about cocaine's effects on behaviour beyond simple ratings of stereotypy intensity and for both day and night portions of the 24-hour day.

4.2. METHODS

4.2.1. Animals, apparatus and drug

Male Sprague Dawley rats (University of Alberta, Ellerslie) weighing 250-350 g on Day 0 of the experiment, were maintained on a 12:12 h light cycle (lights off 0700-1900) with free access to food and water. Each rat was housed individually in a stainless steel cage [26 cm (l) x 24 cm (w) x 16 cm (h)], with a Plexiglas front and wire mesh floor. Animals were pre-assigned to their drug group in a counterbalanced fashion and habituated to these home/test cages for 10 days prior to surgery and behavioural assessment. All subjects were weighed between 0900-1100 every second day throughout the course of the experiment.

All test cages are equipped with photocell beams placed 14 cm apart and 3 cm from the floor on the side walls. Each beam break represents one count and hourly totals are recorded by computer. The sensitivity of these beams was set such that rapid interruptions (less than 1 sec apart) did not register. Therefore, only gross locomotor activity would be counted. In front of each row of cages is an infrared sensitive CCD video camera (Canon Ci-20R) mounted on a truck programmed to move to the next cage at regular video sampling intervals. Each camera comes equipped with an infrared light source (IR-20W) and a 12 mm auto-iris lens (Canon C61205). The video signal is forwarded to a video monitor (Hitachi VM-900), a time date generator (Pelco TDG200DT), and an extended-play video cassette recorder (NEC PV-1200A) situated in an adjacent room.

Alzet^R osmotic minipumps (Alza Corporation, model 2ML2) were filled with either cocaine HCl (BDH) or its vehicle (double-distilled water) according to pump specifications to deliver 0, 2.5, 7.5 or 22.5 mg/day for 14 consecutive days (n=6 per group). These doses were calculated to be approximately equivalent to a single injection of 8.3, 25 or 75 mg/day for an average 300 g rat. All doses were calculated as the salt.

4.2.2. Surgical procedures

Surgery was done between 1300 and 1500 h under sterile conditions after each animal was anesthetized by inhalation with methoxyflurane (Metofane). An incision was made in the midscapular region and a large subcutaneous pocket was created with scissors. The pump was inserted into this pocket which was subsequently sutured with metal clips. The wound was dressed with antibacterial powder and the animal returned to its cage. The pumps were left in place for the entire 2-week period.

4.2.3. Behavioural analysis

On Day 1 behavioural assessment commenced at 1900 h. The video cameras recorded rat activity for the next 24 hours (12 h of light followed by 12 h dark), sampling each rat for 5 min/hour. The same program was run on Day 14 of drug treatment. Locomotor activity, expressed as photocell beam interuptions, was obtained each hour throughout the entire 2-week period. Therefore, other than the regular weighing of the animals, no handling of the animals was necessary.

Video records were rated using the Basic Experimental Behavioural Observation Program (BEBOP) (Martin-Iversen, 1991), a computer program in Turbo Pascal, by an observer blind to the drug treatments. This program measures both the frequency and duration of 28 separate behaviours (Table 4-1) and allows for transitions between pairs of behaviours to be assessed within a given time period. One videotape was coded twice in order to obtain a measure of intraobserver reliability with Hoyt's analysis of reliability (Friedman, 1991).

4.2.4. Statistics

Locomotor activity was assessed as an hourly average for each of the 14 days and nights (12 hours each). These data were subjected to ANOVA with one independent factor (drug treatment) and two dependent factors (day/night and days of treatment).

Behavioural durations and frequencies were totalled for each 12 hour period (Day 1, Night 1, Day 14 and Night 14) and subjected to principal components factor analysis using commercial software (SPSSPC). The resulting three behavioural categories extracted from the factor analysis were subjected to ANOVA with 3 factors: 1) cocaine treatment (coc) with 4 levels (0, 2.5, 7.5, 22.5 mg/day; 2) time of day (day/night) and 3) day of treatment (first/last).

Multivariate tests were applied to ANOVA with repeated measures. These included the Pillais trace, Hotellings T^2, Wilks Lambda and Roys F tests of significance in order to control for homogeneity of variance. Multiple F tests were applied for individual comparisons where appropriate ($p<.05$ significance level). Sequences of behaviours were

Table 4-1. Operational definitions of behaviours assessed in BEBOP.

1	Wet Dog Shake	full body shake, usually begins with upper body and moves to rear torso
2	Eat	gnawing of food pellets
3	Reverse	moving backwards, using all 4 limbs with a result of altering the animal's position in the cage
4	Stretch	extension of the forequarters with the forelimbs stretched out in advance of the animal
5	Yawn	mouth gaping widely
6	Hindlimb Scratch	scratching of the body or head with one of the hindlimbs
7	Pause	a momentary cessation of all active behaviours (e.g. sniffing, walking, but not of postural behaviours)
8	Sniff	rapid flaring and contracting of the nostrils accompanied by movements of the whiskers
9	Drink	licking the water spout
10	Limb Flick	a rapid twitch of one of the limbs
11	Groom	face, head or body stroking with the forelimbs, or licking of any part of the body
12	Head Movement	head weaving (lateral movements) or bobbing (vertical movements)
13	Jump	a leap upwards such that all 4 limbs leave the cage floor at some point
14	Lick	the tongue can be observed making contact with some surface other than the animal's own body
15	Locomote	a forward step taken with each of the 4 limbs that alters the animal's position in the cage
16	Sleep	eyes are closed, breathing is even and slow, animal is immobile and lying down

17	Retrieve Food	reaching into food bin with forepaws or mouth to remove food pellet
18	Contact	snout contact with any part of cage surface
19	Body adjustment	a movement of the trunk of the body that alters the posture of the animal excluding major transitions of posture (i.e. excluding changes between stand, sit, lie, locomote, rear, reverse, jump)
20	Gnaw	chewing on something in the cage other than food or animal's own body
21	Mouth	jaws opening and closing in empty air (excludes gnawing, licking, eating and drinking)
22	Can't Tell	the animal faces away from the camera, occluding observer's view so no adequate assessment available
23	Rear	the animal is standing on its hindlimbs with forelimbs in the air or supporting body against wall
24	Lie	animal is lying down, with legs retracted close to body or stretched out; eyes are open
25	Sit	posture such that animal is sitting on haunches without supporting body with forelimbs
26	Stand	all 4 limbs are extended and apparently supporting the weight of the animal
27	Twitch	sudden quick contraction of some body part
28	Scrabble	repetitive pawing at the floor or wall of cage

analyzed in pairs from the initiation of one behaviour to the initiation of the next using the method of adjusted residuals (Martin-Iverson, 1991).

4.3. RESULTS

4.3.1. Locomotor Activity

Cocaine delivered continuously *via* osmotic minipumps produced changing effects on locomotor activity levels over 14 days and nights. As shown in Fig. 4-1, daytime cocaine-induced locomotion was enhanced in the 7.5 mg/day and 22.5 mg/day cocaine groups on days 2 and 3. With successive days, however, no drug differences were apparent in the high dose group. The locomotor activity in the 7.5 mg/kg group was consistently higher than the other groups, but was significantly greater than vehicle only on days 7 and 13. Nocturnal activity was higher in all animals (including vehicle) relative to daytime activity, and the initial stimulant effect of 7.5 and 22.5 mg/day doses of cocaine were also more robust (Fig. 4-2). Cocaine dose-dependently increased locomotor activity compared to vehicle, with photocell counts doubled on night 1 in the 22.5 mg/day group. Significant increases were still present in the 7.5 and 22.5 mg/day groups after 9 nights. By the 10th night, the stimulant actions of cocaine were diminished to levels no longer different from vehicle controls. The 2.5 mg/day dose served to suppress locomotor activity on the first night, an effect that disappears by Night 2. These results are supported by a significant day/night main effect ($F(1,19)=42.61$, $p<.001$) and day/night by days interaction ($F(13,247)=7.67$, $p<.001$). No significant interaction between cocaine dose and treatment days was found with ANOVA. Chronic cocaine administered to maintain relatively constant

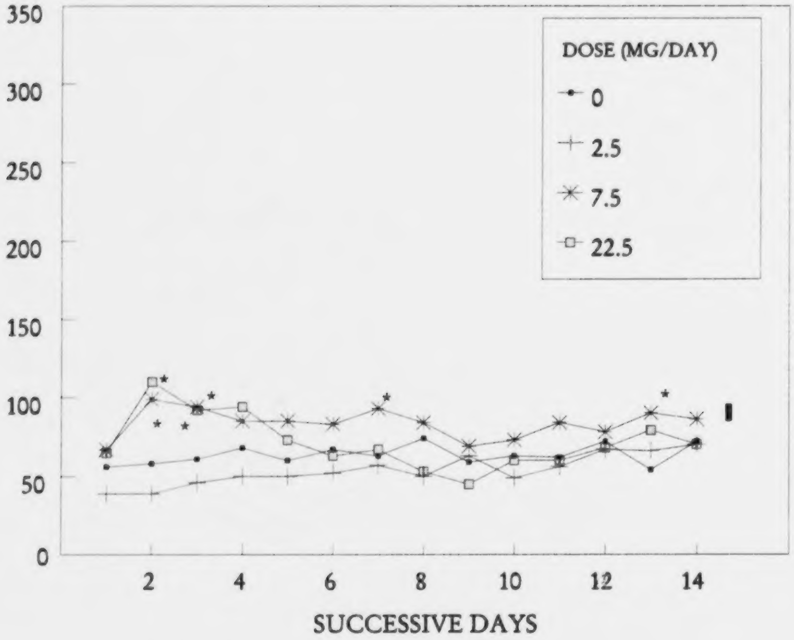

Fig. 4-1. Mean daytime cocaine-induced locomotion as measured by photocell beam interruptions averaged over the 12 hour light cycle for 14 days. Only the two higher doses significantly increased locomotion. Tolerance to these motor stimulant effects of cocaine was seen in both high groups after 4 days of continuous administration. No effect seen in 0 or 2.5 doses, so no tolerance at these doses. Line indicates SE of all means. * Significantly different from vehicle controls.

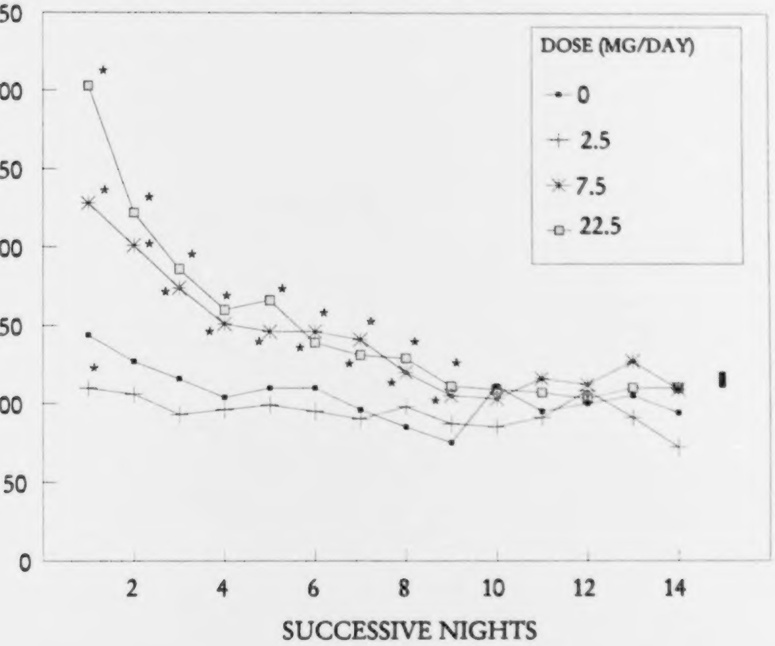

Fig. 4-2. Mean nocturnal activity over 14 successive nights represented as photocell beam interruptions averaged over 12 hours of dark illumination. The mid and high doses of cocaine caused initial stimulant effects which, unlike daytime activity, did not significanlty subside until the 10th day of treatment. Tolerance to the suppressant effects of the 2.5 mg/day dose did, however, appear by Day 2. Line indicates SE of all means. * Significantly different from vehicle controls.

plasma levels over two weeks therefore resulted in reductions in locomotor activity compared to the initial stimulant effects regardless of the day/night cycle.

4.3.2. Behavioural Analysis: Frequencies and Durations

Hoyt's analysis of reliability resulted in intra-observer reliability coefficients of 0.996 (frequencies, standard error of measurement =1.01), 0.999 (durations, standard error of measurement = 3.07 sec) and 0.960 (sequences, standard error of measurement = 1.20).

Principal components analysis of frequencies and durations of 28 separate behaviours revealed 14 factors accounting for approximately 81% of the variance in the behavioural measures. Of these factors, the categories *can't tell*, *eat/retrieve food*, *reverse locomotion*, *drink*, and *lick* were dropped from further analysis due to the rarity of their occurrences. *Pause* was found only to have negative correlations with other behaviors and so was also excluded. The remaining factors were grouped into three major categories (including frequency and duration measures for each behaviour) with the first factor accounting for 31.8% of the variance. This EXPLORE category includes the behaviours *locomote*, *contact*, *rear*, *stand*, *sniff*, *head movement*, *mouth movement*, *gnaw*, *scrabble* and *jump*. The second and third factors (16.1% of the variance) overlapped in that the behavior stretch was included in factor 2 for frequency only and factor 3 for both frequency and duration. Subsequently, these factors were combined to include the INTERMEDIATE behaviours *sit*, *wet dog shake*, *stretch*, *hindlimb scratch*, *limb flick*, *groom* and *yawn*. Finally, the category QUIET was formed to represent two factors (5.6% of the variance) including *sleep*, *lie*, *body adjustment* and *twitch*. These quiet behaviours were also combined on the basis of their high transition frequencies where *sleep* is usually followed by a *body adjustment*. Interestingly, both the *sleep/lie* factor and *pause* category were found to be negatively correlated with the first factor, EXPLORE. This analysis indicates that general exploratory activities can be separated from intermediate behaviours involving specific body parts (i.e. *limb flick, hindlimb scratch, groom* and *yawn*) and quiet behaviours like sleeping or lying down awake.

Frequencies and durations for EXPLORE are shown in Fig. 4-3, with Day 1 and 14 compared to Night 1 and 14. A similar cocaine dose response was seen for both measures, with higher activity levels at night compared to day. As with locomotor activity counts, explore behaviours were decreased in cocaine animals over the two-week period. The mid and high cocaine doses showed stimulant effects in the first 24 hours of drug treatment, but except for the high dose durations, only the night portion was significant. Subsequently, after 14 days and nights EXPLORE activity levels were no different from vehicle in these drug groups. Significant first/last differences were only found at night in the 7.5 and 22.5 mg/day groups. No significant changes in frequency or duration occurred over 14 daytime periods. These findings are supported by a significant drug x first/last x day/night interaction for explore frequencies ($F(3,20)=4.47$, $p<.02$) and significant drug by first/last ($F(3,20)=8.51$, $p<.001$), drug by day/night ($F(3,20)=9.96$, $p<.001$) and first/last by day/night ($F(1,20)=24.24$, $p<.001$) interaction terms for explore durations.

The INTERMEDIATE behavioural category showed different statistical effects for frequency and duration (Fig. 4-4). ANOVA revealed significant main effects of drug ($F(1,20)=3.62$, $p<.05$), first/last ($F(1,20)=39.56$, $p<.001$) and day/night ($F(1,20)=77.57$, $p<.001$) as well as a significant first/last x day/night interaction ($F(1,20)=30.59$, $p<.001$) for

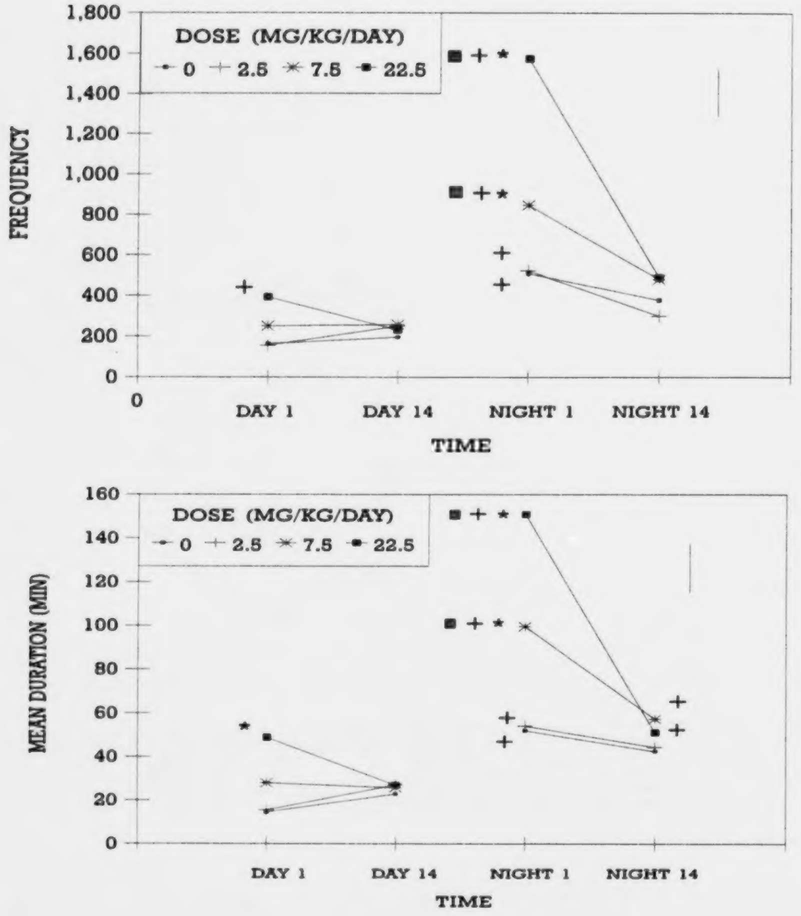

Fig. 4-3. Cocaine dose response for mean frequencies and durations of EXPLORE activity totalled across each 12 hour portion of the first and last days of drug administration. * Significantly different from vehicle controls. Line indicates SE of all means. + Significant day/night difference. ■ Significant first/last difference.

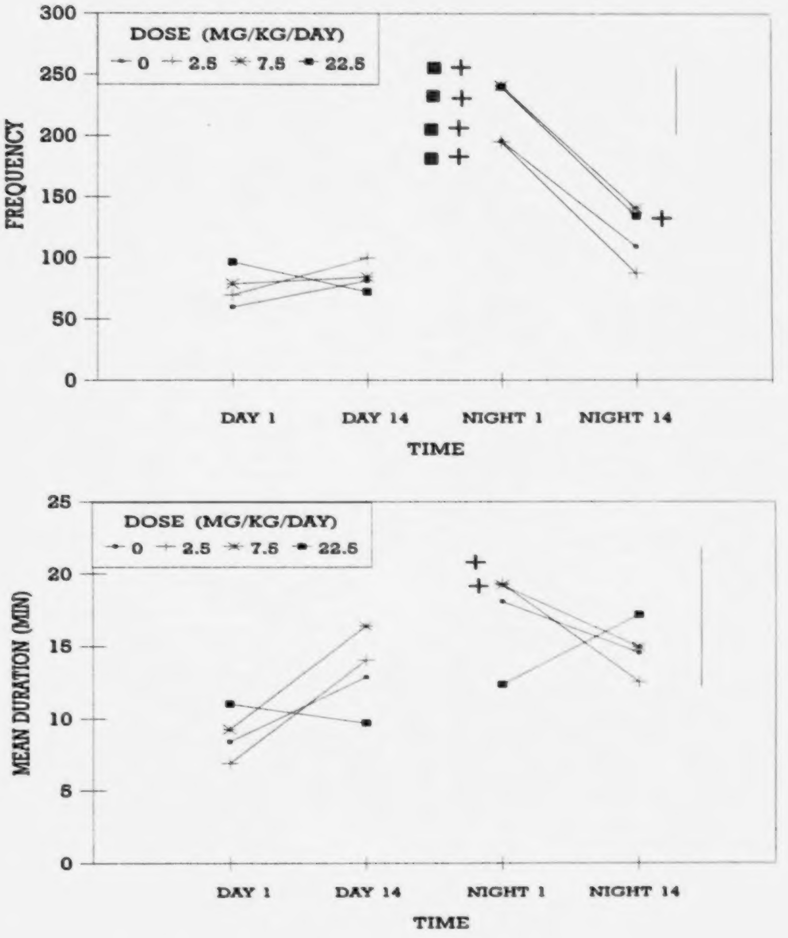

Fig. 4-4. Cocaine dose response for behaviours in the INTERMEDIATE category. Mean frequency and durations totalled for samples taken across 12 hours of day and night on the first and 14th day of treatment. Line indicates SE of all means. + Significant day/night difference. ■ Significant first/last difference.

frequency. Individual comparisons did not reveal any significant increases with any cocaine treatment but nighttime activity levels were significantly greater than daytime for the first 24 hours. After 14 nights, however, INTERMEDIATE frequencies were decreased in all groups, including vehicle. With respect to duration of INTERMEDIATE activity, only a day/night main effect was found (F(1,20)=8.04, p=.01) with day 1 < night 1 for the mid- and high-dose cocaine groups. Therefore, cocaine treatment did not enhance behaviours like *limb flick, groom* or *hindlimb scratch* as a category, and the nocturnal decrease in these behaviours by night 14 was seen for frequency (but not duration) and in all drug groups, including vehicle. No changes occurred during the day.

Sleep, lie, body adjustment and *twitch* were analyzed under the category QUIET activity (Fig. 4-5). Frequency measures show greater activity at night (except for high dose cocaine) with a significant overall day/night effect (F1,20)=11.14, p<.005). Differences between the first and last day of drug treatment are also significant with ANOVA (F(1,20)=9.85, p<.01) but only the 2.5 and 7.5 mg/day cocaine groups showed a significant decrease in the frequency of these behaviours over 14 nights. The interaction between these main effects (first/last x day/night) was also significant with ANOVA (F(1,20)=5.0, p<.05). For QUIET durations, more time was spent engaged in these activities during the light portion of the day/night cycle (F(1,20)=209.49, p<.001). A dose-dependent inverse relationship was found between cocaine and quiet durations such that increasing amounts of drug were found to decrease the time spent sleeping and lying down. This was significant at the 7.5 and 22.5 mg/day level and was supported by an overall main effect of drug (F(1,20)=6.44, p<.01). Both nocturnal and daytime tolerance were found as sleeping

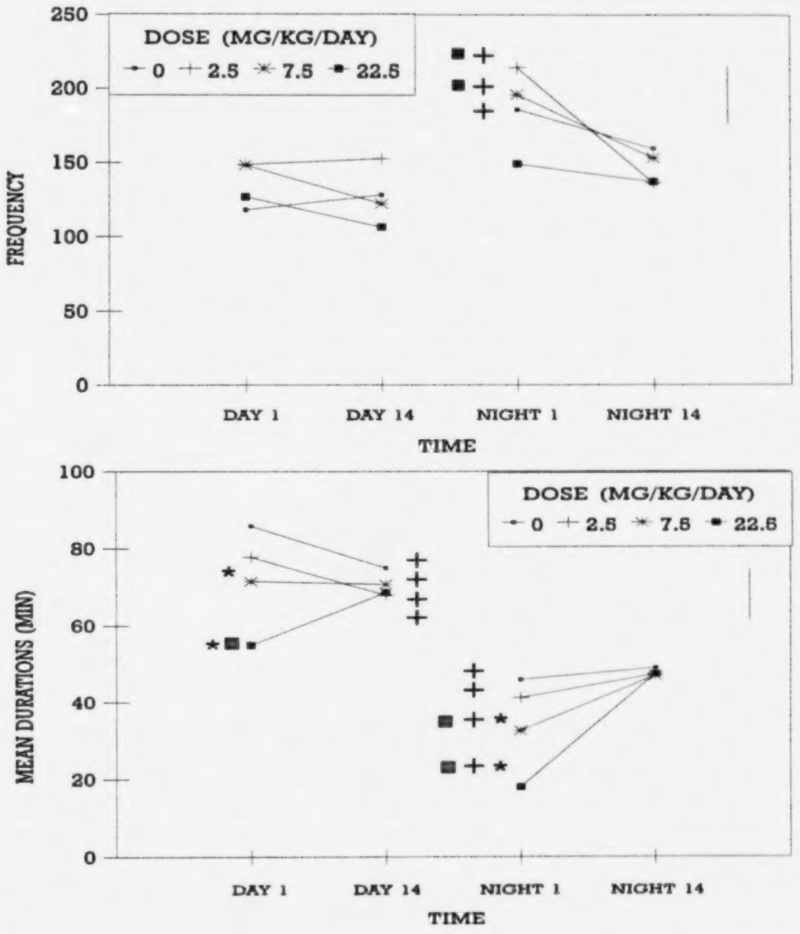

Fig. 4-5. Effect of chronic cocaine on mean frequency and duration of QUIET activity across 12 hours of day and night for two weeks. Line indicates SE of all means. *Significantly different from vehicle controls. + Significant day/night difference. ■ Significant first/last difference.

durations were significantly increased over successive nights (mid and high cocaine groups) and days (high only). These findings are supported by a first/last x day/night interaction for QUIET duration (F(1,20)=11.86, p<.01), but no interaction between cocaine treatment and first/last effects.

4.3.3. Behavioural Transition analysis

An examination of the structure of rat behaviour involved calculating transition ratios for the constituent behaviours of the EXPLORE and QUIET categories only, since no significant effects of cocaine were found for the INTERMEDIATE category. This measure considers the observed transitions between pairs of behaviours that occurred significantly above chance (p<.05) as a function of what was expected based on the frequencies of the behaviours, using the method of adjusted residuals. Since significant drug effects were seen only at night for exploratory activity, in Table 4-2 are transition ratios for the 7.5 and 22.5 mg/day doses of cocaine as well as vehicle animals over 14 nights only. Of the 27 EXPLORE transition pairs, only 7-8 were different in the cocaine groups compared to vehicle on night 1 (i.e. significant after vehicle, but not after cocaine, or *vice versa*). For vehicle animals, the transitions with the highest observed frequency are included in the sequence *locomote* ⇒ *stand* ⇒ *contact* ⇒ *head* ⇒ *mouth* regardless of treatment day. This sequence illustrates movement through the cage followed by standing to explore a particular area of the cage floor. Transitions to behaviours in this sequence were the next highest in frequency and included *sniff* ⇒ *head, sniff* ⇒ *contact* and *mouth* ⇒ *head.* Three short loops were also apparent in these top ten transitions, involving *stand* ⟺ *jump*, *stand* ⟺ *locomote,* and finally *head* ⟺ *mouth*. Exploration while standing therefore involves

Table 4-2. Transition ratios (total observed frequencies /expected) for EXPLORE behaviours in rats treated with vehicle or cocaine for 14 consecutive nights. *Observed transition significantly different than expected transition, p<.05.

Behavioural Transition	Vehicle		Cocaine (7.5 mg/day)		Cocaine (22.5 mg/day)	
	Night 1	Night 14	Night 1	Night 14	Night 1	Night 14
Locomote ⇒ Stand	227/37.2 = 6.1 *	165/4.1 = 6.8 *	494/96.4 = 5.1 *	220/35.9 = 6.1 *	1616/457.2 = 3.5 *	286/54.8 = 5.2 *
Contact ⇒ Head	145/51.1 = 2.8 *	115/46.7 = 2.5 *	272/108.2 = 2.5 *	197/76.4 = 2.6 *	345/128.4 = 2.7 *	186/61.5 = 3 *
Stand ⇒ Contact	122/62.3 = 2.0 *	85/41.2 = 2.1 *	397/191.5 = 2.1 *	150/73.3 = 2 *	863/488.4 = 1.8 *	121/73.5 = 1.6 *
Head ⇒ Mouth	117/43 = 2.7 *	100/39.4 = 2.5 *	105/33.3 = 3.2 *	130/39.6 = 3.3 *	84/23.3 = 3.6 *	88/34.6 = 2.5 *
Jump ⇒ Stand	105/16.9 = 6.2 *	73/9.7 = 7.5 *	197/43.8 = 4.5 *	62/10 = 6.2 *	223/74.6 = 3 *	70/13.5 = 5.2 *
Sniff ⇒ Contact	94/25.9 = 3.6 *	62/18.5 = 3.4 *	193/64.5 = 3 *	71/29.1 = 2.4 *	201/81.2 = 2.5 *	62/22.2 = 2.8 *
Sniff ⇒ Head	89/38.4 = 2.3 *	77/34 = 2.3 *	124/49.4 = 2.5 *	87/38.6 = 2.3 *	131/35.7 = 3.7 *	67/31.1 = 2.2 *
Mouth ⇒ Head	89/43.3 = 2.1 *	69/39.4 = 1.8 *	65/33.4 = 1.9 *	36/39.7 = 0.9	34/23.1 = 1.5 *	62/34.8 = 1.8 *
Stand ⇒ Locomote	84/38.1 = 2.2 *	65/24.8 = 2.6 *	204/98.8 = 2.1 *	92/36.8 = 2.5 *	892/462.5 = 1.9 *	160/56.2 = 2.8 *
Stand ⇒ Jump	67/17.3 = 3.9 *	43/9.9 = 4.3 *	116/44.7 = 2.6 *	36/10.3 = 3.5 *	194/75.4 = 2.6 *	49/13.9 = 3.5 *
Head ⇒ Contact	61/51.2 = 1.2	47/46.4 = 1	104/108.6 = 0.96	67/76.5 = 0.9	155/130.2 = 1.2 *	79/61.9 = 1.3 *
Head ⇒ Sniff	50/38.1 = 1.3 *	43/33.8 = 1.3 *	72/48.5 = 1.5 *	50/38.2 = 1.3 *	45/35.5 = 1.3 *	43/31 = 1.4 *
Contact ⇒ Sniff	39/25.7 = 1.5 *	32/18.6 = 1.7 *	39/63 = 0.6	43/28.8 = 1.5 *	46/79.8 = 0.6	24/22 = 1.1

Contact ⇒ Locomote	31/21.1 = 1.5 *	17/15.4 = 1.1	140/72.8 = 1.9 *	34/28.9 = 1.2	462/277 = 1.7 *	28/33.5 = 0.8
Stand ⇒ Rear	24/13.9 = 1.7 *	20/8.8 = 2.3 *	72/39.3 = 1.8 *	46/17.6 = 2.6	180/100.2= 1.8 *	53/23.1 = 2.3 *
Mouth ⇒ Sniff	24/21.7 = 1.1	9/15.6 = 0.6	40/19.5 = 2.1 *	17/14.9 = 1.1	83/14.3 = 5.8 *	18/12.4 = 1.5 *
Rear ⇒ Contact	17/7.9 = 2.2 *	17/5.5 = 3.1 *	78/29.3 = 2.7 *	47/14 = 3.4 *	129/61.6 = 2.1 *	61/14.6 = 4.2 *
Rear ⇒ Sniff	11/5.9 = 1.9 *	8/4.0 = 2 *	11/13.1 = 0.8	3/7 = 0.4	18/16.8 = 1.1	12/7.3 = 1.6 *
Gnaw ⇒ Head	7/1.3 = 5.4 *	4/1.8 = 2.2 *	0/0.1 = 0	2/1.3 = 1.5	0	0
Jump ⇒ Rear	6/2.2 = 2.7 *	2/1.3 = 1.5	18/15.1 = 1.2 *	4/2 = 2.0	35/9.4 = 3.7 *	9/2.7 = 3.3 *
Contact ⇒ Scrabble	3/0.5 = 6.0 *	0	5/1.1 = 4.5 *	4/2 = 2.0	25/6.2 = 4.0 *	0
Scrabble ⇒ Contact	2/0.5 = 4.0 *	0	2/1.1 = 1.8	9/1.9 = 4.7 *	11/6.3 = 0.2 *	1/0.4 = 2.5
Mouth ⇒ Gnaw	2/0.8 = 2.5	1/0.8 = 1.3	0/0 = 0	0/0.6 = 0	0	2/0.5 = 4.0 *
Gnaw ⇒ Sniff	2/0.7 = 2.9 *	3/0.7 = 4.3 *	0/0.1 = 0	1/0.5 = 2	3/0.4 = 7.5 *	4/0.5 = 8 *
Gnaw ⇒ Mouth	1/0.8 = 1.3	1/0.8 = 1.3	1/0 = n/a *	5/0.5 = 10 *	2.0/0.2 = 10 *	2/0.5 = 4 *
Contact ⇒ Gnaw	1/1 = 1	4/1 = 4 *	0/0.1 = 0	2/1.1 = 1.8	6/1.4 = 4.3 *	5/0.9 = 5.5 *

making snout contact with the cage surface, sniffing, head and mouth movements or simply jumping up or moving forward without further investigation of the area.

On night 1, when significant drug effects are reported in this category, cocaine increased the observed transitions through the same basic sequence seen in vehicle animals. Essentially, cocaine did not alter the underlying pattern of behaviour but only increased the traffic through these exploratory transitions. On the other hand, the ratios of observed:expected transitions were not increased. This indicates that there was not an increased switching between a decreased number of behaviours, but rather simply an increase in the initiation of behaviour. For the 7.5 mg/day group, only 2 of the top 10 transitions were different from vehicle. *Contact* ⇒ *locomote* and *stand* ⇒ *sniff* were increased at the expense of the *head* ⟺ *mouth* loop. The high dose cocaine animals exhibited 3 sequence changes in their top ten list. Again *contact* ⇒ *locomote* was added along with *stand* ⇒ *rear* and *head* ⇒ *contact*. The same short loop between *head* and *mouth* was decreased even further with this higher cocaine dose and *sniff* ⇒ *head* was also no longer a major transition. Transitions frequencies were generally higher for the high dose cocaine group compared to 7.5 mg/day except for *mouth* ⇒ *head* which exhibited an inverse relationship with increasing cocaine dose. *Jump* ⇒ *stand*, *sniff* ⇒ *contact* and *sniff* ⇒ *head* transitions were increased to approximately the same degree regardless of cocaine dose. Other transitions involving the behaviours *mouth, gnaw,* and *sniff* were not significantly affected by cocaine treatment with respect to observed frequencies but the ratios calculated in Table 4-2 show that these transitions did occur more often than expected in the 22.5 mg/day cocaine group. These changes in the frequency of transitions suggests that cocaine produces a dose-dependent heightened exploration involving switching from forward movement to standing (or *vice versa*) and making snout contact with the cage surface. Scanning the area *via* head movements follows this sequence but, unlike vehicle, is less likely, with increasing dose of cocaine, to be followed by any movement of the mouth (and *vice versa*).

In Table 4-3, sequence analysis of QUIET activity illustrates the nocturnal habits of normal vehicle-treated rats. Essentially, the most frequent transitions in this category were between *twitch* ⟺ *body adjustment* ⟺ *lie* ⇒ *sleep* regardless of treatment day. The 7.5 mg/day group did not alter the frequencies of any QUIET transitions except for slight decreases in *sleep, twitch* and *body adjustment* interactions. High dose cocaine, however, decreased frequencies in all but *lie* ⇒ *body adjustment* on the first night of treatment. In additon, 2 of the 9 transitions were no longer significant for this particular drug group. An interesting finding involved the sequence *sleep* ⇒ *body adjustment* which was increased above expected levels with high dose cocaine and may be an indication that sleep, although decreased in this drug group, was probably fitful and interrupted by slight body movements. Cocaine treatment did not alter the basic sequence of QUIET behaviours seen in vehicle animals; it only decreased the frequency of transitions within it.

After 14 nights of cocaine treatment, EXPLORE activity is no longer increased above vehicle animals and activity levels are, for the most part, equivalent across groups. Sequence changes are also restored to those seen in vehicle. *Contact* ⇒ *locomote, stand* ⇒ *rear* and *stand* ⇒ *sniff,* transitions greatly increased with cocaine, are now replaced by the same transitions they displaced (*head* ⟺ *mouth, sniff* ⇒ *head).* QUIET frequencies are

Table 4-3. Mean transition ratios (observed/expected) for QUIET behaviours in rats treated with vehicle or cocaine (22.5 mg/day) for 14 consecutive nights. *Observed transition significantly different from expected transition, p<.05.

Behavioural Transition	Vehicle		Cocaine (7.5 mg/day)		Cocaine (22.5 mg/day)	
	Night 1	Night 14	Night 1	Night 14	Night 1	Night 14
Twitch ⇒ Body adj.	81/32.0 = 2.5 *	76/26.9 = 2.8 *	71/27.6 = 2.6 *	60/23.6 = 2.5 *	19/6.5 = 2.9 *	36/16.8 = 2.1 *
Body adj. ⇒ Twitch	62/31.9 = 1.9 *	46/26.3 = 1.7 *	54/27.1 = 2 *	44/23.6 = 1.9 *	13/6.6 = 2.0 *	25/16.5 = 1.5 *
Body adj. ⇒ Lie	23/7.4 = 3.1 *	24/11.1 = 2.2	26/10.3 = 2.5 *	16/5.9 = 2.7 *	11/6.5 = 1.7 *	18/6.6 = 2.7 *
Lie ⇒ Sleep	18/0.8 = 22.5 *	18/0.7 = 25.7 *	11/0.3 = 36.7 *	23/0.6 = 38.3 *	2.0/0.1 = 20 *	19/0.5 = 38 *
Lie ⇒ Body adj.	17/11.5 = 1.5 *	25/17.3 = 1.4 *	23/13.1 = 1.8 *	15/11.7 = 1.3	13/6.9 = 1.9 *	13/10.5 = 1.2
Sleep ⇒ Body adj.	15/3.3 = 4.5 *	11/3.4 = 3.2 *	8/1.3 = 6.1 *	11/2.6 = 4.2 *	1.0/0.1 = 10 *	8.0/2.1 = 3.8 *
Body adj. ⇒ Sleep	13/3.9 = 3.3 *	8.0/4.4 = 1.8 *	8/1.7 = 4.7 *	6/3.8 = 1.6	0	5.0/2.7 = 1.9
Sleep ⇒ Twitch	12/1.7 = 7.1 *	6.0/0.9 = 6.7 *	4/0.5 = 8 *	6/0.8 = 7.5 *	1.0/0 = 0 *	6.0/0.7 = 8.6 *
Sleep ⇒ Lie	2.0/0.4 = 5 *	2.0/0.4 = 5 *	1/0.2 = 5 *	0/0.2 = 0	0	1.0/0.3 = 3.3

now increased in those transitions previously reduced by cocaine treatment. Essentially, after two weeks, high dose cocaine-treated animals have activity levels and patterns more like vehicle-treated animals.

4.4. DISCUSSION

The present experiment supports previous research that tolerance to cocaine's locomotor and stereotypy-inducing properties occurs when the drug is administered by continuous infusion. Unlike the psychomotor stimulants amphetamine (Martin-Iverson and Iversen, 1989) and PHNO (Martin-Iverson et al., 1988a,b), however, this locomotor tolerance occurs at night as well as during the day. The nocturnal sensitization seen with other stimulant drugs was not a feature of continuous cocaine treatment. This finding was also reported for slightly higher cocaine doses in mice over 14 days and nights (Reith et al., 1987). When exploratory behaviours were considered as a whole, both frequency and duration measures showed significant decreases after chronic administration of cocaine. Tolerance to the stimulant nature of cocaine was also illustrated by increases in quiet activity over the 2-week treatment period. Sleeping and lying down durations were collectively increased in a dose-dependent fashion and were significantly higher after 14 days and nights in the 22.5 mg/day group. These findings not only highlight the importance of the day/night cycle in determining stimulant effects on behaviour but also demonstrate the differences between several different psychmotor stimulants on behaviour.

The finding of decreased locomotor activity after 2 weeks of continuous cocaine administration regardless of day/night cycle is different from other psychomotor stimulants in a similar drug administration paradigm. Amphetamine, a DA agonist which increases the release of DA and can therefore affect a number of DA receptor subtypes, has been shown to induce tolerance during the day but sensitization at night (Martin-Iverson and Iversen, 1989). The D2 receptor agonist PHNO follows a similar pattern (Martin-Iverson et al., 1988a) but with a more robust sensitization effect as measured by photocell beam interruptions with daily injections of the drug (Martin-Iverson and McManus, 1990). This effect of PHNO has been shown to be dependent on concomitant stimulation of D1 receptors since co-treatment with a D1 antagonist blocks the initial locomotor stimulation effects of PHNO and a D1 agonist reverses daytime tolerance to the D2 agonist (Martin-Iverson et al., 1988a). These results led to the hypothesis that locomotor sensitization was related to D1 receptor supersensitivity with differential results depending on the circadian rhythms in endogenous DA levels. Since cocaine is thought to block the reuptake of DA (Heikkila et al., 1975; Ross and Renyi, 1967) thereby increasing synaptic levels of this neurotransmitter, more endogenous DA is available to bind to DA receptors which correlates with increased locomotor activity (Kuczenski and Segal, 1989; Carboni et al., 1989; Kalivas and Duffy, 1990). The action of all three of these psychomotor stimulants on the DA system is, therefore, ultimately the same: increased DA neurotransmission. There are, however, several differences between these drugs with respect to specific effects on the DA system. PHNO is selective for D2 receptors while amphetamine and cocaine affect DA levels in the synpatic cleft and can therefore influence binding to any DA receptor subtype. Amphetamine induces release of DA and, at higher doses, like cocaine, blocks reuptake. These different mechanisms of action may therefore be responsible for the differences in the expression or structure of this hyperactive response. Sensitization to amphetamine at night may be mediated by the DA release-enhancing properties of this drug, a mechanism of action not associated with continuous cocaine treatment. Increased stimulated DA release has been correlated with sensitization to amphetamine (Antelman et al., 1980; Castaneda et al., 1988; Robinson and Becker, 1982; Robinson et al., 1982, 1988) but, to date, no work has shown that this effect does not occur in treatment regimens that produce tolerance. Another possibility stems from cocaine's ability to affect impulse activity in DA neurons. Administration of cocaine has been shown to suppress unit activity of DA containing cells in the SN (Pitts and Marwah, 1987, 1987) and VTA (Pitts and Marwah, 1986, 1987; White et al., 1987; Einhorn et al., 1988; White et al., 1992). With repeated administration *via* daily intermittent injections, cocaine induces behavioural sensitization which has been shown to be accompanied by increased impulse activity in these cell body regions (Henry et al., 1989). With continuous adminstration, as in the present study, it may be that cocaine continues to dampen the impulse activity in DA neurons to prevent the nocturnal sensitization seen with other DA agonists. Evidence supporting this stems from findings of a lack of increased extracellular DA after continuous cocaine delivery in the NAS (King et al., 1992) as well as reports of no change in DA receptor occupation in the striatum and NAS (see Chapter 5).

Finally, a consideration of the local anesthetic properties of cocaine may provide an explanation for the differences in locomotor response over the circadian cycle in comparison with amphetamine and PHNO. Cocaine delivered continuously may lead to changes in the processes that mediate its local anesthetic actions in such as way so as to prevent sensitization of DA cell activity and behaviour. More research is needed in order to fully understand the precise differences between amphetamine and cocaine mechanisms on the DA system and how they relate to behaviour.

A further look at the structure of behaviour involved an examination of the transitions between behaviours in the EXPLORE and QUIET categories. These results show that initially, the frequency of explore transitions is increased with increasing doses of cocaine, while decreasing the occurrence of transitions in the QUIET category. In other words, general activity is enhanced in a dose-response fashion. This heightened activity is achieved not by the formation of new behavioural transitions, but rather by increasing the frequency of specific actions already present in the animal's behavioural repertoire. This is supported by the finding that only 8 of 27 possible EXPLORE transitions were significantly different in high dose cocaine- *versus* vehicle-treated animals in the first 24-hour exposure period. The basic EXPLORE and QUIET sequences seen in vehicle-treated animals were not much altered with cocaine treatment. After two weeks of treatment, transition frequencies were returned to a level not unlike that of vehicle controls.

The psychomotor stimulants have largely been associated with the development of stereotypy with increasing doses. This perseverance in responding, according to the Lyon and Robbins (1975) hypothesis, is described as a decrease in the number of overall response categories due to behavioural competition between sequences (Robbins and Sahakian, 1983). In other words, certain behaviours are lost due to increased traffic through other sequences that are strenghthened through repetition. An examination of QUIET activity in the present study shows that these transitions are decreased in frequency with high dose cocaine treatment, as is the number of observed transitions (from 9 to 7 on night 1). It therefore seems apparent that stereotypy occurs as a result of increases in EXPLORE transition frequencies at the expense of sequences involving *sleep* and *lie* which are significantly reduced (and even eliminated) by high dose cocaine. More importantly to a dose-response analysis of behavioural competition, however, is an analysis of the competition between locomotor effects of cocaine seen at lower doses and the emergence of oral stereotypy at higher doses. In this experiment, locomotor activity was not decreased at the expense of oral activity with high dose cocaine since these behaviours were positively correlated with each other in the EXPLORE behavioural category. Similarly, transitions involving locomote were not decreased in high dose cocaine; they increased with the oral transitions in observed frequency.

The concept of behavioural competition is also illustrated by enhanced switching between behaviour sequences as well as abortive response sequences as behaviours drop out of the sequence. In the present study, the number of significant transitions was not greatly affected by cocaine treatment, and, overall, the pattern of behaviour was not much altered. The proportion of observed:expected transitions was not increased in prepotent behaviours (i.e. behaviours determined by rate of occurrence for baseline or vehicle activity) as compared to less prepotent behaviours, as would be predicted by the behavioural competition hypothesis. The most frequent transitions in EXPLORE or QUIET were virtually the same for vehicle- and cocaine-treated animals, regardless of treatment day. The top transitions for vehicle formed a sequence from *locomote* ⟺ *stand* ⇒ *contact* ⇒ *head* ⟺ *mouth*. The most noticeable change in this pattern was the emergence of more *contact* ⇒ *locomote* and *stand* ⇒ *sniff* or *rear* transitions on the first night of cocaine treatment. The repetitive loop of *locomote* ⇒ *stand* ⇒*contact* ⇒ *locomote* and a shorter *stand* ⟺ *locomote* version was therefore much more likely to occur with increasing doses of cocaine. These loops may illustrate the stereotypical nature of cocaine-induced behaviour as they are repetitive in nature and may be considered abortive versions of longer sequences. The increase in these transitions with cocaine were not generally accompanied by increases in the ratio of observed:expected frequencies of transitions. Instead, *locomote* ⇒ *stand*, actually decreased suggesting that cocaine increases the initiation of behaviour. Expected frequencies are based on the frequency of initiations of stand and locomote, for example, in this dyad. Only a decrease in the frequency of expected transitions would indicate that these behaviours are initiated less often and that the response categories have therefore decreased. Cocaine therefore does not result in an increase in switching between fewer behaviours; instead the same categories show increased frequency. After 2 weeks of cocaine treatment, these sequences return to the same pattern as in vehicle-treated rats. With respect to the theory of behavioural competition with psychomotor stimulants, it would appear that cocaine induces perseverative behaviour through increased traffic in existing behaviour patterns without drastically altering the underlying structure to any degree.

With regard to the intermediate behaviours, unlike either of the other behavioural categories, video sampling of frequency and duration showed no significant effect of chronic cocaine. *Limb flick, wet dog shake, groom, hindlimb scratch, stretch, yawn* and *sit*, as a collective category was not significantly enhanced by any cocaine dose at any point measured on the first and last days of drug treatment. Although these behaviours occurred less often after 14 nights of cocaine treatment, the same results were found in untreated animals. Since all subjects, regardless of treatment, showed the same trend after 2 weeks, it would appear that no competition between these behaviours occurs with increasing cocaine dose. In addition, these findings do not support previous work with chronic cocaine which suggests that this particular psychomotor stimulant is an effective inducer of hallucinogenic-type behaviours like limb flicks, wet dog shakes and parisitotic grooming with prolonged exposure (Ellison, 1991). These late stage emergent behaviours have been proposed as a model of stimulant-induced psychosis, a behavioural state not unlike the active psychosis of paranoid schizophrenia (Ellison, 1991). Based on the results reported here, it would appear that continuous infusion of cocaine does not always produce these aberrant behaviours in greater frequency than in vehicle-treated animals. Unlike the present study, however, Ellison delivered cocaine *via* subcutaneous pellet implants which do not function to release drug in the same manner as osmotic minipumps (Eison et al., 1983). It is therefore possible that the regimen of administration was different enough to produce different behavioural effects. It is also important to note that these particular actions are very low frequency behaviours and the time-sampling procedure used in the present experiment may have been inadequate to assess these behaviours.

In summary, it appears that cocaine has a different behavioural profile than other psychomotor stimulants in that continuous infusion leads to locomotor tolerance (measured by photocell beam interruptions) regardless of the day/night cycle. Tolerance to cocaine's effects on exploratory and quiet behaviours as measured with video sampling techniques, is produced with chronic treatment with greater initial stimulant effects at night compared to the day. This change in behaviour is represented as a shift from perseverative stereotypy-like activities to explore sequence patterns similar in frequency to normal vehicle-treated animals after two weeks of cocaine treatment. No major changes in the underlying structure of these behaviours were found with cocaine. Finally, the lack of a measurable cocaine effect on behaviours such as *wet dog shake* and *limb flick* suggests that this particular method of continuous drug administration (i.e. osmotic minipumps) may not be a useful model of stimulant-induced psychosis.

4.5. REFERENCES

Angrist, B. (1983) Psychosis induced by central nervous system stimulants and related drugs. In: Creese I (eds) *Stimulants: neurochemical, behavioral and clinical perspectives.* Raven Press, New York, pp 1-30.

Antelman, S.M., Eichler, A.J., Black, C.A. and Kocan, D. (1980) Interchangeability of stress and amphetamine in sensitization. *Science, 207:*329-331.

Carboni, E., Imperato, A., Perezzani, L. and Di Chiara, G. (1989) Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. *Neurosci., 28:*653-661.

Castaneda, E., Becker, J.B., and Robinson, T.E. (1988) The long-term effects of amphetamine treatment in vivo on amphetamine, KCl and electrical stimulation evoked striatal dopamine release in vitro, *Life Sci.*, *42*: 2447-2456.

Damianopoulos, E.N. and Carey, R.J. (1993) Apomorphine sensitization effects: Evidence for environmentally contingent behavioural reorganization processes. *Pharmacol. Biochem. Behav., 45:*655-663.

Damianopoulos, E.N. and Carey, R.J. (1992) Conditioning, habituation and behavioral reorganization factors in chronic cocaine effects. *Behav. Brain. Res. 49:*149-157.

Einhorn, L.C., Johansen, P.A. and White, F.J. (1988) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J. Neurosci., 8:*100-112.

Eison, M.S., Eison, A.R. and Iversen, S.D. (1983) Two routes of continuous amphetamine administration induce different behavioural and neurochemical effects in the rat. *Neurosci. Lett., 39:*313-319.

Ellison, G.D. (1991) Animal models of hallucinations. In: Boulton A.A., Baker G.B., Martin-Iverson M.T. (eds) *Neuromethods, Vol 18: Animal Models in Psychiatry I.* The Humana Press Inc., Totowa, N.J. pp 151-195.

Friedman, P. (1991) GB-STAT™ Version 3.0. *Computer aided statistics and graphics tutorial/manual.* Dynamic Microsystems Inc., Silver Spring MD.

Henry, D.J., Greene, M.A. and White, F.J.(1989) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: repeated administration. *J. Pharmacol. Exp. Ther., 251:*833-839.

Heikkila, R.E., Orlansky, H., and Cohen, G. (1975) Studies on the distinction between uptake inhibition and release of ^{3}H-dopamine in rat brain tissue slices. *Biochem. Pharmacol., 24:* 848-852.

Kalivas, P.W. and Duffy, P. (1990) The effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse, 5:*48-58.

King, G.R., Joyner, C., Lee, T., Kuhn, C., and Ellinwood Jr, E.H. (1992) Intermittent and continuous cocaine administration: Residual Behavioral states during withdrawal. *Pharmacol. Biochem. Behav. 43:*243-248.

Kuczenski, R. and Segal, D. (1989) Concomitant characterization of behavioural and striatal neurotransmitter response to amphetamine using *in vivo* microdialysis. *J. Neurosci., 9:*2051-2065.

Lyon, M. and Robbins, T.W. (1975) The action of central nervous system stimulant drugs:

A general theory concerning amphetamine effects. In: Essman, W. and Valzelli, L. (eds) *Current developments in psychopharmacology, vol 2*. Spectrum, New York, pp.79-163.

Martin-Iverson, M.T. (1991) An animal model of stimulant-induced psychoses. In: Boulton, A.A., Baker, G.B. and Martin-Iverson, M.T. (eds) *Neuromethods, Vol 19, Animal models in psychiatry I.* Humana Press, New Jersey, pp. 103-149.

Martin-Iverson, M.T. and McManus, D.J. (1990) Stimulant-conditioned locomotion is not affected by blockade of D1 and/or D2 dopamine receptors during conditioning. *Brain Res., 521:*175-184.

Martin-Iverson, M.T. and Iversen, S.D. (1989) Day and night locomotor activity effects during administration of (+)-amphetamine. *Pharmacol. Biochem. Beh. 34:*465-471.

Martin-Iverson, M.T., Iversen, S.D., Stahl, S.M. (1988a) Long-term motor stimulant effects of (+)-4-propyl-9-hydroxynaphthoxazine (PHNO), a dopamine D2 receptor agonist: Interactions with a dopamine D1 receptor antagonist and agonist. *Eur J Pharmacol 149:*25-31.

Martin-Iverson, M.T., Stahl, S.M. and Iversen, S.D. (1988b) Chronic administration of a selective dopamine D2 agonist: factors determining behavioral tolerance and sensitization. *Psychopharmacol. 95:* 534-539.

Pitts, D.K. and Marwah, J. (1986) Electrophysiological effects of cocaine on central monoaminergic neurons. *Eur. J. Pharmacol., 131:*95-98.

Pitts, D.K. and Marwah, J. (1987) Cocaine modulation of central monoaminergic neurotransmission. *Pharmacol. Biochem. Behav., 26:*453-461.

Post, R.M., Weiss, S.R.B., Fontana, D. and Pert, A. (1992) Conditioned sensitization to the psychomotor stimulant cocaine. *Ann. N. Y. Acad. Sci. 654:*386-399

Reith, M.E.A., Benuck, M., Lajtha, A. (1987) Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. *J. Pharmacol. Exp. Ther. 243:*281-287.

Robbins, T.W., and Sahakian, B.J. (1983) Behavioral effects of psychomotor stimulant drugs: Clinical and neuropsychological implications. In: Creese, I. (ed) *Stimulants: Neurochemical, behavioral, and clinical perspectives*. Raven Press, New York, pp 301-338.

Robbins, T.W., Mittleman, G., O'Brien, J. and Winn, P. (1990) The neuropsychological significance of stereotypy induced by stimulant drugs. In: Cooper, S.J. (ed) *Neurobiology of stereotyped behaviour*. Oxford University Press, London, pp.25-63.

Robinson, T.E. and Becker, J.B. (1982) Behavioural sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro, *Eur. J. Pharmacol.*, *85*: 253-254.

Robinson, T.E., Becker, J.B. and Presty, S.K. (1982) Long-term facilitation of amphetamine-induced rotational behaviour and striatal dopamine release produced by a single exposure to amphetamine: sex differences, *Brain Research*, *253*: 231-241.

Robinson, T.E., Jurson, P.A., Bennett, J.A. and Bentgen, K.M. (1988) Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)amphetamine: a microdialysis study in freely moving rats. *Brain Research*, *462*: 211-222.

Ross, S.B. and Renyi, A.L. (1967) Inhibition of the uptake of tritiated catecholamines by antidepressant and related agents. *Eur. J. Pharmacol., 2:*181-186.

Segal, D.S., and Schuckit, M.A. (1983) Animal models of stimulant induced psychosis. In: Creese, I.(ed) *Stimulants: neurochemical, behavioral and clinical perspectives.* Raven Press, New York, pp 131-168

White, F.J., Wachtel, S.R., Johansen, P.A., and Einhorn, L.C. (1987) Electrophysiological studies of the rat mesoaccumbens dopamine system: focus on dopamine receptor subtypes, interactions and the effects of cocaine. In: Chiodo, L.A. and Freeman, A.S. (eds) *Neurophysiology of dopaminergic systems-current status and clinical perspectives.* Lakeshore Publishing, Detroit, pp. 317.

White, F.J., Henry, D.J., Hu, X.T., Jeziorski, m. and Ackerman, J.M. (1992) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system. In: Lakoski, J.M., Galloway, M.P. and White, F.J. (eds) *Cocaine: pharmacology, physiology and clinical strategies,*CRC Press, Boca Raton, Fl., pp.261-294.

CHAPTER 5. BEHAVIOURAL SENSITIZATION AND TOLERANCE TO COCAINE AND THE OCCUPATION OF DOPAMINE RECEPTORS BY DOPAMINE[1]

5.1. INTRODUCTION

Psychomotor stimulant drugs, including cocaine, have been associated with different behavioural effects in rats depending on the regimen of administration. Repeated daily injections produces sensitization, a gradual increase in drug-induced locomotion and other behaviours such as sniffing, rearing and head movements (Angrist, 1983; Segal and Schuckit, 1983). This intermittent drug delivery has been proposed as an animal model of stimulant-induced psychosis (Angrist, 1983; Segal and Schuckit, 1983). Continuous infusion, on the other hand, leads to the development of behavioural tolerance, even with doses equivalent to those producing sensitization (Kokkinidis, 1984; Martin-Iverson et al., 1987, 1988; Post, 1980). Sensitization is therefore a function of factors related to the treatment regimen.

Several neurochemical substrates have been proposed to account for sensitization. As cocaine is thought to act at the DA transporter to block uptake into presynaptic terminals, its effect is to enhance DA neurotransmission *via* increasing DA levels in the extracellular space (Hurd and Ungerstedt, 1989; Kalivas and Duffy, 1990; Pettit et al., 1990). The development of behavioural sensitization with repeated administration has thus been associated with many different aspects of the DA system, including changes in pre- and/or post-synaptic receptor density, neurotransmitter synthesis, metabolism and spontaneous release (see Johanson and Fischman, 1989; Robinson and Becker, 1986 for reviews on this area). However, the most consistent finding is one of an increase in DA release stimulated by KCl, electrical stimulation or amphetamine, observed in both *in vitro* release and *in vivo* microdialysis studies (Antelman et al., 1980; Castaneda et al., 1988; Robinson and Becker, 1982; Robinson et al., 1982, 1987, 1988). This research is purely correlational, however, and does not convincingly establish a role for enhanced stimulated DA release in sensitization to either cocaine or amphetamine. In addition, there are several discrepancies in the *in vivo* microdialysis research related to the withdrawal period such that enhanced extracellular DA does not always accompany behavioural sensitization during early withdrawal (Hurd et al., 1989; Segal and Kuczenski, 1992a,b). Repeated administrations of stimulants to animals produce a variety of effects in a variety of brain regions, any one (or more) of which may be responsible for behavioural sensitization.

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[1] This chapter formed the basis for two manuscripts. A version has been published (Burger and Martin-Iverson, 1994, *Brain Res., 639:*228-232) and a separate version has been accepted for publication (Martin-Iverson and Burger, in press, *Mol. Neurobiol.*).

The hypothesis that behavioural sensitization results from increased DA release after cocaine would be strengthened if rats given cocaine continuously, to produce tolerance, fail to exhibit increased stimulated DA release. Additional support would also come from a treatment regimen involving a pharmacological blockade of sensitization that also blocks increased DA release. Research using the drug nimodipine, an L-type calcium channel antagonist, has previously demonstrated its ability to block sensitization (Reimer and Martin-Iverson, 1994) and the impulse-dependent increase in extracellular DA concentrations by cocaine (Pani et al., 1990). The present experiment was designed to fulfill both of these conditions by measuring the occupation of DA receptors in animals given treatment regimens with cocaine that produce sensitization, tolerance or the pharmacological blockade of sensitization with nimodipine. A comparison was made between receptor occupation levels on the last day of treatment and following a single cocaine challenge after a 10 day withdrawal period in order to account for any possible dispositional effects of cocaine in the two treatment regimens. More importantly, this study utilized a new approach to indirectly measure DA release by assessing the amount of DA that binds to its receptors. This is achieved *via* the compound N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) which acts by binding to DA receptors to irreversibly denature them *via* alkylation. Because it has a relatively moderate affinity for the receptor complex, only those sites not bound with DA (or any other ligand with high affinity) are vulnerable to EEDQ denaturation (Hamblin and Creese, 1983; Meller et al., 1985; Saller et al., 1989). Therefore, previously occupied receptor sites are left intact for measurement with standard binding assays. This method provides information about the relative amount of DA bound to its receptors following a given treatment regimen and has been shown to be sensitive to the effects of cocaine at elevating the occupation of D1 and D2 receptors (Burger and Martin-Iverson, 1993).

5.2. METHODS

5.2.1. Animals

Male Sprague-Dawley rats (Charles River Canada) weighing 250-350 g were individually housed in colony rooms maintained on a 12 hour light-dark cycle (lights on at

0800 h) at 22°C. Food and water was available *ad libitum*. All subjects were weighed every second day throughout the course of the experiment.

5.2.2. Drug Administration Procedure

After 10 days of habituation following arrival, the rats were randomly assigned to one of 6 drug groups consisting of treatments of vehicle or nimodipine and vehicle or cocaine (VEH+VEH, VEH+COCi, VEH+COCc, NIM+VEH, NIM+COCi, NIM+COCc, where VEH is vehicle, COCi is intermittent cocaine, COCc is continuous cocaine and NIM is nimodipine). Cocaine hydrochloride (BDH, 10 mg/kg/day) and vehicle (double distilled water) were delivered either continuously *via* Alzet^R osmotic minipumps (Alza, model 2ML2) or intermittently with daily intraperitoneal (IP) injections to half of the rats for 14 days. Surgical implantation of the minipumps (method described in Burger and Martin-Iverson, 1993, see also Chapter 3) was performed on Day 0. Nimodipine (Miles Pharmaceuticals Ltd., 10 mg/kg) or its vehicle, polyethylene glycol 400 (100%), was injected IP 70 min prior to locomotor testing each day, beginning on Day 1. Immediately following the daily cocaine injection (vehicle was injected for those animals with minipumps and for the vehicle controls) each rat was placed in its own box equipped with photocell beams (as described previously in Chapter 4) to assess activity levels for one hour. On Day 14, 30 minutes post-cocaine or vehicle, 8 subjects in each group were administered EEDQ (5 mg/kg, IP) dissolved in ethanol, propylene glycol and distilled water (2:1:2). The remaining 72 rats (N: VEH+VEH = 20, VEH+COCi = 14, VEH+COCc = 14, NIM+VEH = 8, NIM+COCi = 8, NIM+COCc = 8) remained in their home cages for 10 additional days. All but 6 rats in the VEH+VEH group were then injected with cocaine (10 mg/kg, IP), placed in the locomotor testing cages and 30 min later were given an injection of EEDQ as above. Six of the rats from the VEH+VEH group were given a challenge injection of vehicle, to serve as controls. Rats were observed periodically to determine whether or not they engaged in high levels of stereotyped behaviours that might confound the locomotor measurements. Twenty-four hours after the EEDQ injections, the rats were decapitated and their brains removed for dissection on ice (see Chapter 2 for procedure). Left and right sides of the striatum and NAS were removed and stored at -80°C for further neurochemical analysis.

5.2.3. DA Receptor Binding

Unilateral striatal tissue from each rat (left and right sides randomized across assays) was subjected to radioligand binding assays for both D1 and D2 receptor labelling as previously described (see Chapter 2). [^{3}H]SCH23390 and [^{3}H]spiperone were used to label D1 and D2 receptors, respectively, with the addition of ketanserin to control for $5HT_2$ receptor binding. Due to the small amount of tissue obtained with dissection of the NAS, only single point binding for D1 and D2 receptor assessment was performed. Ligand concentrations were 0.678 nM for D1 and 0.049 nM for D2 receptor analysis (based on the respective K_d values for each receptor). All ligands and assay procedures were otherwise the same as for the striatal binding.

5.2.4. Statistics

The 5-minute blocks of photobeam interruptions were added together to produce 30 min totals for the last day of treatment before EEDQ injection (pre-withdrawal) or on the challenge test day (post-withdrawal). These data as percent of the vehicle control groups were then subjected to ANOVA with three factors. These factors were: 1) withdrawal having two levels (0 or 10 days); 2) nimodipine treatment with two levels (vehicle or 10 mg/kg); and 3) cocaine treatment with three levels (vehicle, 10 mg/kg/day intermittent injections or 10 mg/kg/day continuous infusions). Receptor densities (Bmax) and affinities (K_d) were calculated from the data from the receptor binding assays using LIGAND and were subjected to ANOVA with the three factors listed above after calculating the percentage of vehicle controls (the vehicle challenge group was the control in the case of the post-withdrawal groups). The D1 and D2 receptor subtypes were included as an additional within-subject factor. Due to procedural difficulties, NAS binding data was only obtained for the pre-withdrawal group which was subjected to a seperate ANOVA for nimodipine, cocaine and continuous *versus* intermittent treatment effects. Multiple F tests (Kiess, 1989) were applied for individual comparisons where appropriate. Pearson's multiple correlations analysis between locomotor counts and DA receptor binding results were also calculated.

5.3 RESULTS

5.3.1. Pre-Withdrawal

Pre-withdrawal locomotor activity is displayed in Figure 5-1 for the 13 consecutive days of drug treatment prior to EEDQ administration. Intermittent cocaine treatment increased locomotor activity over the 2-week period whereas continuous delivery produced initial stimulatory effects that dissipated quickly to locomotor levels similar to untreated animals. Daily nimodipine pre-treatment attenuated the behavioural effects of intermittent cocaine. Intermittent daily injections of cocaine substantially increased locomotor response on the last day of treatment before the day of EEDQ injections (Fig. 5-2). Continuous

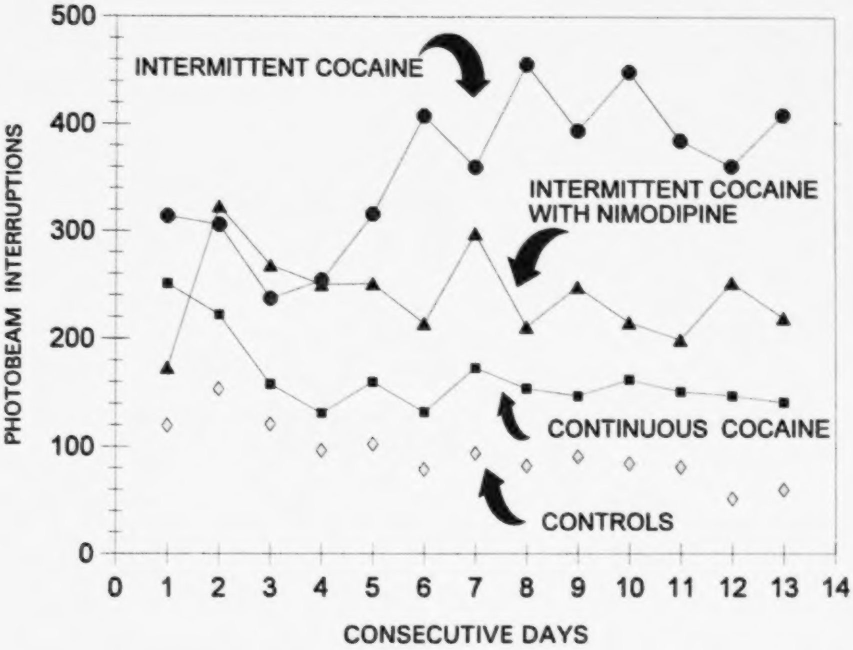

Fig. 5-1. Locomotor activity levels expressed as mean photocell beam interruptions for 60 minutes post-treatment over successive days. For the sake of clarity, vehicle groups (intermittent and continuous), nimodipine with vehicle and nimodipine with continuous cocaine rats were pooled together as controls, since there were no significant differences among these groups in terms of activity levels.

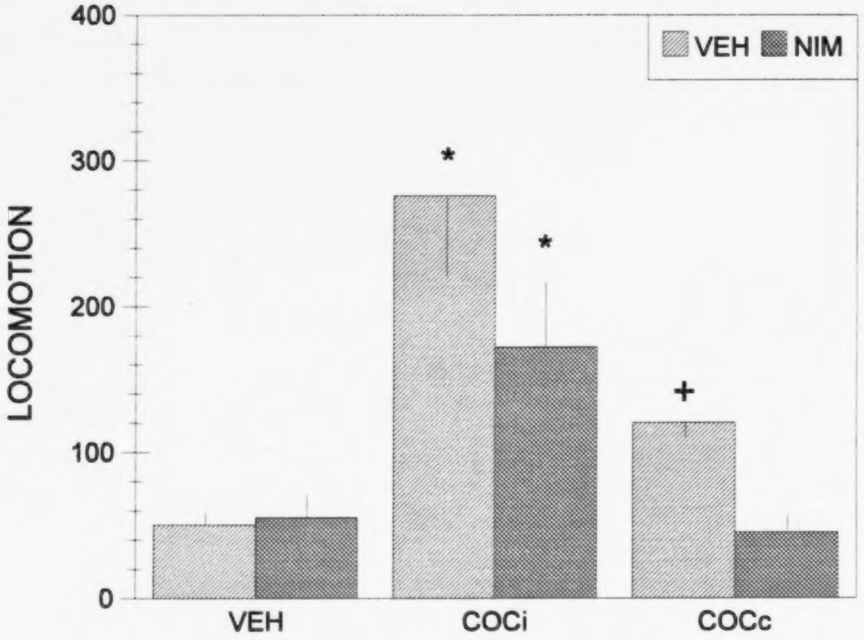

Fig. 5-2. Mean locomotor activity (± SEM) in rats (n=8 in each group) for 30 min on the last day of treatment prior to EEDQ injection before withdrawal from treatment. Rats were treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/day) and vehicle (VEH), intermittent daily injections of cocaine (COCi) or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg. Pairwise comparisons between groups were made with the Multiple F test for $p < .05$. *Significantly different from vehicle control. + Significantly different from the COCi group.

infusions of cocaine produced locomotor activity that was not significantly different from the vehicle control group, but was significantly lower than that of the intermittent cocaine group, indicating the development of tolerance in the continuous infusion group. Nimodipine attenuated the increase in locomotor activity produced by intermittent cocaine such that this group was intermediate between the intermittent cocaine and the vehicle groups. ANOVA revealed that there were main effects of cocaine treatment regimen ($F(2,102)=25.6$, $p < 0.001$), of withdrawal ($F(1,102)=6.5$, $p < 0.02$), and of nimodipine ($F(1,102)=5.39$, $p < 0.05$). The withdrawal by cocaine treatment interaction was also significant ($F(2,102)=6.31$, $p < 0.005$). Striatal receptor K_d values observed in each group were assessed by ANOVA. There were no significant effects of any of the treatments. The K_d for D1 receptors was 0.32 ± 0.01 (nM ± SEM) and for D2 receptors was 0.053 ± 0.0017 (nM ± SEM). However, the densities of receptors remaining after denaturation by EEDQ differed markedly between groups, depending on treatment. The average densities of DA receptors remaining in the vehicle control group after denaturation of receptors by EEDQ were 384 fmol/mg protein (D1) and 123 fmol/mg protein (D2). There were significant main effects of withdrawal ($F(1,102)=6.74$, $p < 0.02$), and nimodipine ($F(1,102) = 3.22$, $p\ 0.02$) for the striatal data. There was also a significant interaction between nimodipine and cocaine treatment regimen ($F(2,102)=3.89$, $p < 0.025$) and between withdrawal and receptor subtype ($F(1,102)=7.80$, $p < 0.01$). A significant interaction between nimodipine, cocaine and treatment regimen was also observed for DA receptors in the NAS ($F(1,40)=5.78$, $p < .05$). In the pre-withdrawal groups, only the group receiving intermittent cocaine injections exhibited an increased occupation of D1 (Fig. 5-3 and 5-5) and D2 receptors (Fig. 5-4 and

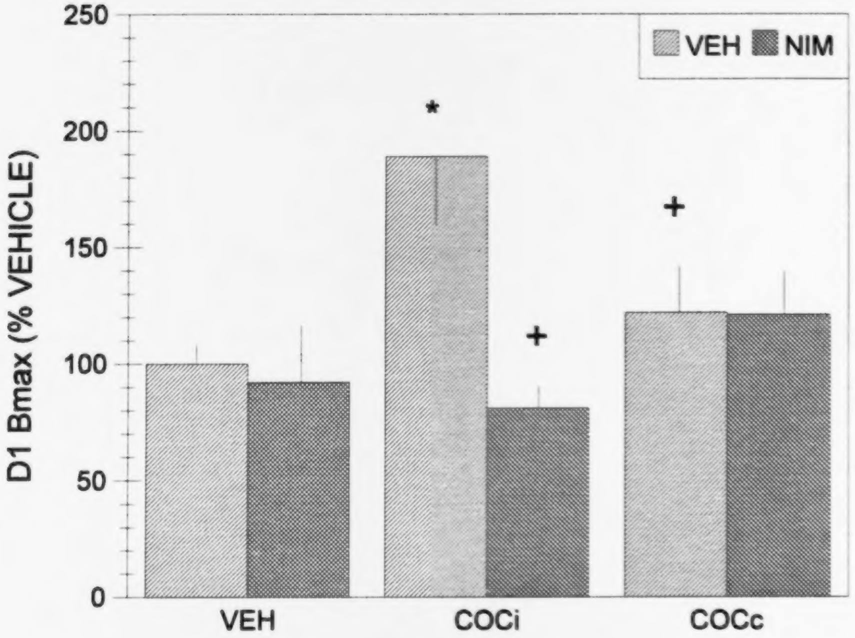

Fig. 5-3. The density of DA D1 receptors (mean ± SEM) protected from EEDQ denaturation on the last day of treatment as a percent of the vehicle control group. Rats were treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/day) and vehicle (VEH), intermittent daily injections of cocaine (COCi) or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg and n=8 for each group . Pairwise comparisons between groups were made with the Multiple F test for $p < .05$. * Significantly different from vehicle control. + Significantly different from the COCi group.

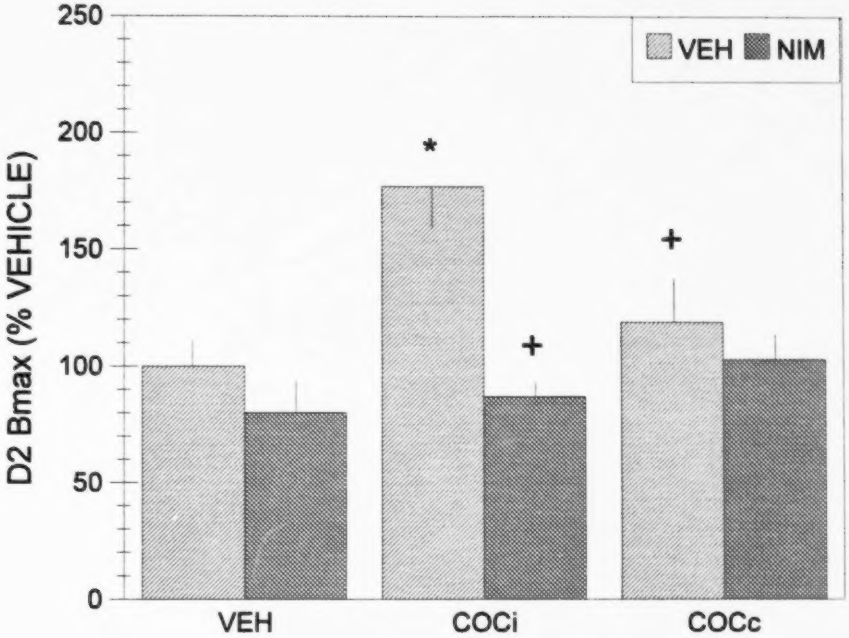

Fig. 5-4. The density of DA D2 receptors (mean ± SEM) protected from EEDQ denaturation on the last day of treatment as a percent of the vehicle control group. Rats were treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/day) and vehicle, intermittent daily injections of cocaine (COCi) or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg, and n=8 for each group. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. * Significantly different from vehicle control. + Significantly different from the COCi group.

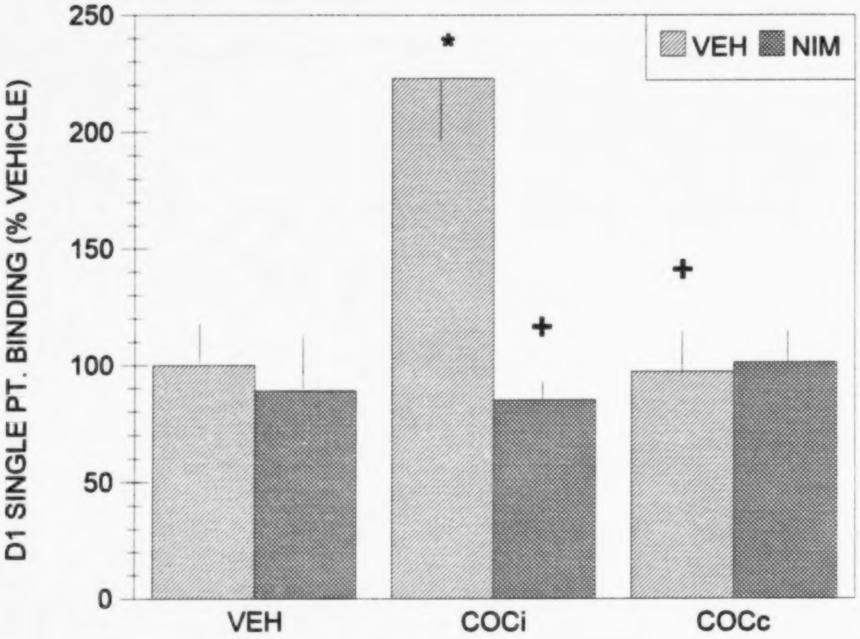

Fig. 5-5. The density of DA D1 receptors (mean ± SEM) in the NAS 24 hours post-EEDQ injection on Day 14 of pre-withdrawal. Data are represented as percent of vehicle control. * significantly different from controls, p<.05.

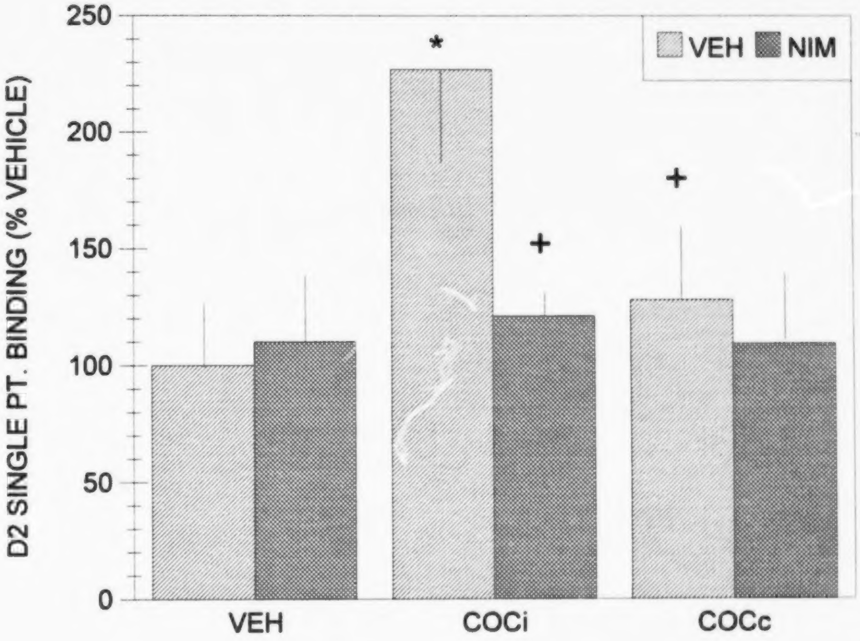

Fig. 5-6. The density of DA D2 receptors (mean ± SEM) in the NAS 24 hours post-EEDQ injection. Data are represented as percent of vehicle control. * Significantly different from controls, p<.05.

5-6). The groups receiving continuous infusions of cocaine did not show significant elevations in the protection of DA receptors from EEDQ-induced denaturation. Nimodipine given prior to intermittent cocaine injections blocked the effects of cocaine on both striatal and accumbens receptor occupancy. Correlational analysis of behaviour with receptor binding revealed a significant correlation between locomotor activity and increased occupation of D1 (but not D2) receptors in the striatum ($r=0.41$, $p <.01$). In the NAS, however, locomotion correlated with D2 (but not D1) receptor occupation (rr= 0.34, $p<.05$).

5.3.2.Post-Withdrawal

The effects of daily drug administration on rats subjected to a period of withdrawal are shown in Fig. 5-7. As with the first pre-withdrawal group, intermittent but not continuous cocaine increased locomotion above control levels over the 10 day period. Again, this increase was attenuated by nimodipine. After 10 days of withdrawal (Fig. 5-8), cocaine challenge given to rats with a history of intermittent cocaine resulted in locomotion levels significantly greater than that observed in the vehicle group receiving a cocaine challenge. This effect was not altered by nimodipine, and was not observed in the groups previously given continuous infusions with cocaine.

The group that received previous treatment with vehicle and was challenged with cocaine exhibited an increase in the occupation of both striatal D1 (Fig. 5-9) and D2 (Fig. 5-10) receptors. This increase in the occupation of receptors induced by an acute cocaine injection was blocked in those rats with a history of treatment with nimodipine. Intermittent treatment with cocaine increased the occupation of D1 and D2 receptors over that observed

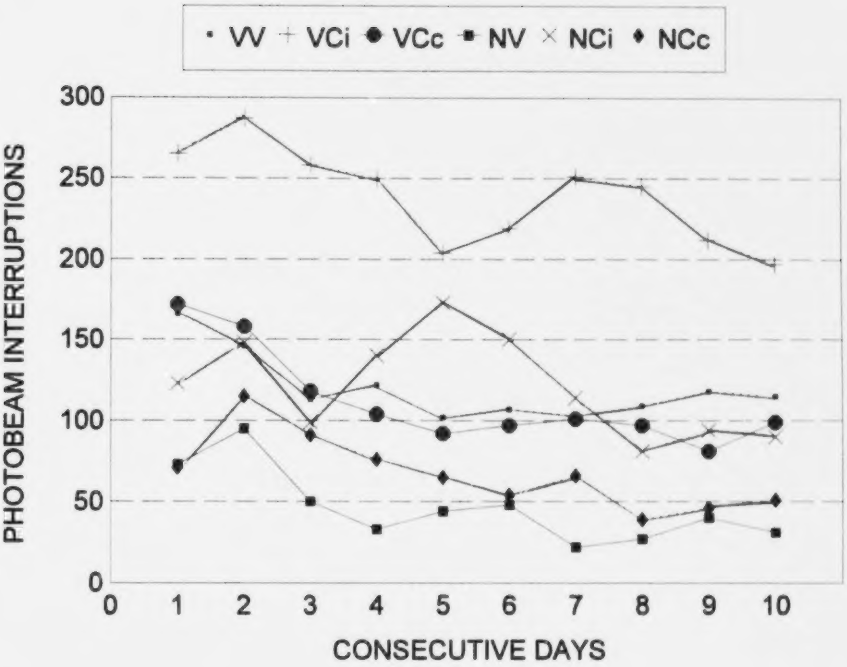

Fig. 5-7 Mean locomotor activity measured as frequency of photobeam interruptions over the 10 days of drug treatment prior to withdrawal. Data are from the first 30 minutes of each day including the last day (Day 10) prior to EEDQ adminstration.

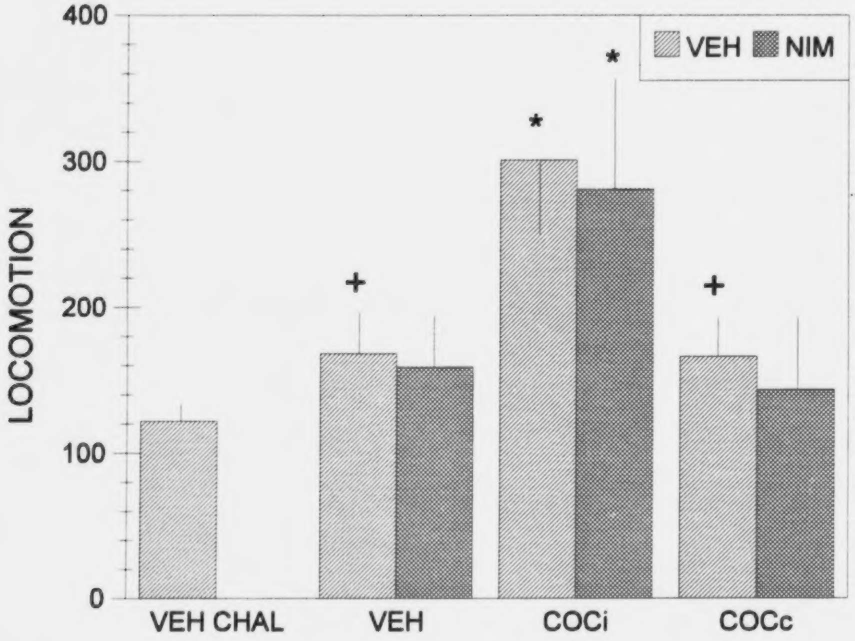

Fig. 5-8. Locomotor activity (mean ± SEM) in rats for 30 min after a cocaine (10 mg/kg) challenge 10 days after withdrawal from a chronic treatment regimen. A control group was given a vehicle challenge 10 days after withdrawal from vehicle treatment (VEH CHAL, n=6). Rats were chronically treated with vehicle or nimodipine (NIM, 10 mg/kg/day) and vehicle, intermittent daily injections of cocaine (COCi) or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg, N=14 for all cocaine challenged groups that did not receive nimodipine, and n=8 for all groups receiving nimodipine. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. * Significantly different from vehicle control. + Significantly different from the COCi group given a cocaine challenge.

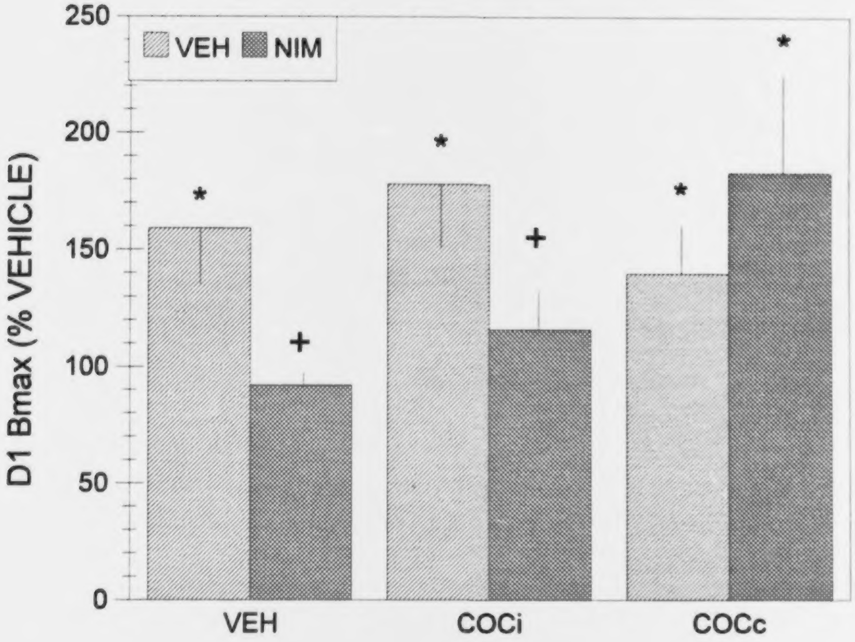

Fig. 5-9. The density of DA D1 receptors (mean ± SEM) following a cocaine (10 mg/kg) challenge 10 days after withdrawal from a chronic treatment regimen as a percent of the vehicle control group. Rats were chronically treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/day) and vehicle, intermittent daily injections of cocaine (COCi) or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg, n=14 for all open bar groups and n=8 for all groups represented by cross-hatched bars. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. * Significantly different from vehicle control. + Significantly different from the COCi group given a cocaine challenge.

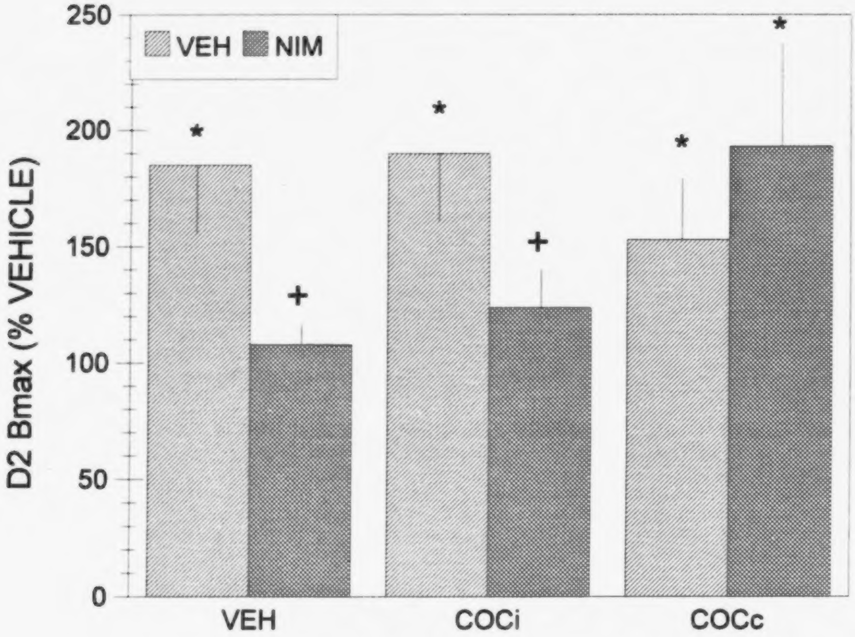

Fig. 5-10. The density of DA D2 receptors (mean ± SEM) following a cocaine (10 mg/kg) challenge 10 days after withdrawal from a chronic treatment regimen as a percent of the vehicle control group. Rats were chronically treated with vehicle (VEH) or nimodipine (NIM, 10 mg/kg/day) and vehicle, intermittent daily injections of cocaine (COCi) or continuous infusions of cocaine (COCc). The cocaine daily dose was 10 mg/kg, n=14 in all groups represented by open bars, and n=8 in all groups represented by cross-hatched bars. Pairwise comparisons between groups were made with the Multiple F test for $p < 0.05$. * Significantly different from vehicle control. + Significantly different from the COCi group given a cocaine challenge.

in the vehicle challenged rats but not over that observed in the acute cocaine group. Again, previous treatments with nimodipine blocked the effects of the cocaine challenge in this group. Continuous infusions of cocaine slightly, but not significantly, attenuated the increase in both D1 and D2 receptor occupation after a challenge injection of cocaine as compared to the intermittent treatment group. The occupation of receptors in the continuous group tended to be less than the vehicle group challenged with a cocaine injection, but not significantly so. However, prior co-administration of nimodipine had no significant effect on altering the response to cocaine challenge in the continuous cocaine group, and in fact had a tendency to increase occupation.

Correlational analysis for the post-withdrawal group failed to reveal any significant correlations between behaviour and striatal D1 or D2 receptor occupancy. No data were obtained for NAS binding in this group.

5.4. DISCUSSION

Intermittent daily treatment of rats with cocaine increased locomotion significantly greater than that observed in rats given continuous infusions of cocaine or vehicle injections. In addition, co-treatment of rats during the 14 days of intermittent cocaine treatment with nimodipine (an L-type calcium channel antagonist) attenuated cocaine-induced locomotion. Both of these effects have been reported previously (Burger and Martin-Iverson, 1994; Post, 1980; Reimer and Martin-Iverson, 1994). The present experiment also determined whether these differences in the stimulant effects of cocaine are associated with differences in the occupation of DA D1 and D2 receptors by DA in the striatum. Rats receiving intermittent injections of cocaine and exhibiting sensitized levels of locomotion also have an increase in the occupation of both D1 and D2 receptor sites (Figures 5-3-6; Burger and Martin-Iverson, 1994). However, rats that received the same dosage of cocaine on a daily basis, but *via* continuous infusions with osmotic minipumps did not exhibit changes in the occupation of DA receptors. Thus, rats exhibiting tolerance to cocaine had no more DA occupying receptor sites than those given vehicle.

Nimodipine not only attenuated the behavioural effects of intermittent cocaine, it also blocked the cocaine-induced increase in occupation of striatal and NAS DA D1 and D2 receptors (Figures 5-3 to 5-6, respectively and Burger and Martin-Iverson, 1994). This is consistent with a previous report that nimodipine blocks the increase in extracellular DA produced by cocaine (Pani et al., 1990). That the decrease in cocaine-induced locomotion produced by either continuous infusions of cocaine or treatment with nimodipine are both associated with decreases in the occupation of DA receptors increases confidence that this association may be of a causal nature.

The rats given a 10 day withdrawal period after chronic drug administration were subsequently given a single cocaine challenge prior to EEDQ. The intermittent cocaine group exhibited sensitization, i.e. higher levels of locomotion than the vehicle group given a challenge. The continuous infusion group did not exhibit differences from the acute cocaine-challenged vehicle group or from a vehicle-challenged control group. Prior treatment with nimodipine had no effect on locomotion in rats that had been withdrawn from nimodipine treatment for 10 days.

The cocaine challenge increased the occupation of both D1 and D2 receptors in the vehicle, intermittent cocaine and continuous cocaine groups (see Figs 9 and 10). Therefore, previous history of different treatment regimens did not affect the occupation of D1 and D2 receptors in the striatum. Nimodipine blocked the increase in occupancy in both the vehicle and the intermittent groups. However, it had no significant effects on receptor occupancy when given to rats treated with continuous cocaine, and in fact exhibited a nonsignificant trend towards increasing protection of the receptors from denaturation by EEDQ.

The two different patterns of effects observed pre- and post-withdrawal indicate that the effects on receptor occupancy observed pre-withdrawal are likely a function of differences in brain levels of cocaine at the time of EEDQ treatment between the two treatment regimens. Reith et al. (1987), have shown that treatment history does not influence brain cocaine levels with a cocaine challenge after a period of withdrawal. If the receptor occupancy was related to behavioural sensitization, then a similar pattern of receptor occupancy effects should have been observed pre- and post-withdrawal, since sensitization occurred in the intermittent treated groups under the two conditions. In addition, significant correlations between behaviour and receptor occupancy should have been obtained for both periods of withdrawal. For the striatum, D1 receptor occupancy and locomotion were correlated pre- but not post-withdrawal. Thus, we observed a dissociation between the behavioural and the receptor occupancy effects of withdrawal from the intermittent treatment regimen.

A second dissociation between the behaviour and the receptor occupancy was observed in the nimodipine-treated groups. Prior treatment with nimodipine had no effects on locomotion produced by a challenge dose of cocaine 10 days after cessation of treatment.

However, previous nimodipine treatment still reduced the occupation of DA receptors in the group with no previous cocaine history, and in the group that had a history of intermittent injections of cocaine. Nimodipine did not affect DA receptor occupation in the continuous cocaine group suggesting that this treatment regimen may lead to reductions in Ca^{2+}-mediated processes such that nimodipine would have no effect. As an L-type Ca^{2+} channel antagonist, nimodipine's greatest effects are during heightened levels of depolarization (Silverstone and Grahame-Smith, 1992). It is therefore possible that tolerance may be related to decreased depolarization of DA cell bodies.

These results indicate that behavioural sensitization is not a function of increased DA release in the striatum. This result is similar to those of some microdialysis studies of amphetamine sensitization (Kuczenski and Segal, 1990; Segal and Kuczenski, 1992a) and of cocaine (Hurd et al., 1989; Segal and Kuczenski, 1992b). There are a several microdialysis studies that have found increases in extracellular DA in the NAS (ventral striatum) of rats exhibiting behavioural sensitization to cocaine (Akimoto et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990) and amphetamine or methamphetamine (Akimoto et al, 1990; Kazahaya et al., 1989; Patrick et al., 1991; Robinson et al., 1988). Kalivas and Duffy (1993) may have resolved the reasons for these differences. They found that there was no relation between extracellular DA levels in the accumbens and sensitization after a challenge dose of cocaine after short withdrawal periods but an apparent relation at longer withdrawal periods. Especially relevant to the present experiment, they did not find significant increases in the area under the curve for DA determinations over 120 min after a cocaine challenge 7 or 10 days post-withdrawal. It is clear that Kalivas and Duffy dissociated behavioural sensitization from accumbens DA levels in time-course, as we have dissociated sensitization from occupation of striatal DA receptors. It remains possible that alterations of DA release in other regions, such as the substantia nigra, VTA or extra-accumbens limbic areas are the critical changes underlying sensitization and tolerance in the locomotor effects of cocaine. This possibility is consistent with previous studies that suggest that behavioural sensitization is associated with DA D1 receptor effects in the DA cell body regions (Stewart and Vezina, 1989; Vezina, 1993; Vezina and Stewart, 1990), and with those that indicate that conditioned effects of cocaine alter *fos* expression, an indicator of differential gene activation which has been shown to be dependent on D1 receptor stimulation (Graybiel et al., 1990), in limbic areas other than the NAS (Brown et al., 1992). Interestingly, nimodipine not only blocks L-type Ca^{2+} channels which are located primarily on cell bodies, but also inhibits D1 receptor-mediated behavioural effects (Kostowski et al., 1990) possibly *via* its inhibition of cAMP (Pucilowski, 1992). Sensitization to cocaine may therefore be mediated by alterations in D1 intracellular transduction mechanisms, a finding also suggested by increased cAMP activity in the NAS (Terwilliger et al., 1991), which may in turn be blocked with nimodipine co-treatment.

In summary, the locomotor stimulant effects of cocaine were found to be associated with changes in the occupancy of DA D1 and D2 receptors with a treatment regimen that produces behavioural sensitization increasing occupancy, and a regimen that produces tolerance decreasing receptor occupancy. Nimodipine, an L-type calcium channel blocker, decreases both the behavioural and receptor occupancy effects of cocaine. However, a cocaine challenge after a 10-day withdrawal period dissociated the behavioural effects from those on receptor occupancy in the striatum. Behavioural sensitization and tolerance to cocaine were both associated with an increase in the occupation of striatal D1 and D2 receptors of a similar magnitude as after a single acute injection of cocaine. These results indicate that while DA release in the striatum or NAS may be sufficient for behavioural sensitization, it is not necessary. If the amount of DA interacting with its receptors is necessary in determining sensitization and tolerance, then it must occur in brain regions other than the striatum.

5.5 REFERENCES

Akimoto, K., Hamamura, T. and Otsuki, S. (1989) Subchronic cocaine treatment enhances cocaine-induced dopamine efflux: studies by *in vivo* intracerebral dialysis. *Brain Res. 490*: 339-344.

Akimoto, K., Hamamura, T., Kazahaya, Y., Akiyama, K. and Otsuki, S. (1990) Enhanced extracellular dopamine levels may be the fundamental neuropharmacological basis of cross-behavioural sensitization between methamphetamine and cocaine - an *in vivo* dialysis study in freely moving rats. *Brain Res. 507*: 344-346.

Angrist, B. (1983) Psychoses induced by central nervous system stimulants and related drugs, In Creese, I. (Ed), *Stimulants: Neurochemical, Behavioural, and Clinical Perspectives*, Raven Press, New York, pp. 1-30.

Antelman, S.M., Eichler, A.J., Black, C.A. and Kocan, D. (1980) Interchangeability of stress and amphetamine in sensitization, *Science*, *207*: 329-331.

Brown, E.E., Robertson, G.S. and Fibiger, H.C. (1992) Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. *J. Neurosci., 12:* 4112-4121.

Burger, L.Y. and Martin-Iverson, M.T. (1993) Day/night differences in D1 but not D2 DA receptor protection from EEDQ denaturation in rats treated with continuous cocaine, *Synapse*, *13*: 20-29.

Burger, L.Y. and Martin-Iverson, M.T. (1994) Increased occupation of D1 and D2 dopamine receptors accompanies cocaine-induced behavioural sensitization. *Brain Res., 639:* 228-232.

Castaneda, E., Becker, J.B., and Robinson, T.E. (1988) The long-term effects of amphetamine treatment in vivo on amphetamine, KCl and electrical stimulation evoked striatal dopamine release in vitro, *Life Sci.*, *42*: 2447-2456.

Graybiel, A.M., Moratalla, R. and Robertson, H.A. (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. *Proc. Natl. Acad. Sci., 87:*6912-6916.

Hamblin, M.W. and Creese, I. (1983) Behavioural and radioligand binding evidence irreversible dopamine receptor blockade by N-ethoxycarbonyl-2-ethoxy-1,2--dihydroquinoline, *Life Sci.*, *32*:2247-2255.

Hurd, Y.L. and Ungerstedt, U. (1989) Cocaine: an in vivo microdialysis evaluation of its acute action on dopamine transmission in rat striatum, *Synapse*, *3:* 48-54.

Hurd, Y.L., Weiss, F., Koob, G.F. and Ungerstedt, N (1989) Cocaine reinforcement and extracellular dopamine overflow in rat nucleus accumbens: an *in vivo* microdialysis study. *Brain Res. 498*: 199-203.

Johanson, C.-E. and Fischman, M.W. (1989) The pharmacology of cocaine related to its abuse, *Pharmacol. Rev.*, *41*: 3-52.

Kalivas, P.W. and Duffy, P. (1990) Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens, *Synapse, 5*: 48-58.

Kalivas, P.W. and Duffy, P. (1993) Time course of extracellular dopamine and behavioural sensitization to cocaine. I. dopamine axon terminals. *J. Neurosci. 13:* 266-275.

Kazahaya, Y., Akimoto, K. and Saburo, O. (1989) Subchronic methamphetamine treatment enhances methamphetamine- or cocaine-induced dopamine efflux *in vivo*. *Biol.*

Psychiat. 25: 903-912.

Kiess, H.O. (1989) *Statistical Concepts for the Behavioural Sciences*. Allyn and Bacon, Boston, pp.326-331.

Kokkinidis, L. (1984) Effects of chronic intermittent and continuous amphetamine administration on acoustic startle, *Pharmacol. Biochem. Behav.*, *20*: 367-372.

Kostowski, W., Krzascik, P. and Pucilowski, O. (1990) Effect of calcium channel inhibitors on D-1 receptor-mediated responses: SKF 38393-induced grooming and SCH 23390-induced catalepsy in rats. *Biogenic Amines, 7*:49-56.

Kuczenski, R. and Segal, D.S. (1990) *In vivo* measures of monoamines during amphetamine-induced behaviour in rat. *Prog. Neuropsychopharmacol. Biol. Psychiat. 14*: S37-S50.

Martin-Iverson, M.T., Stahl, S.M. and Iversen, S.D. (1988) Chronic administration of a selective dopamine D2 agonist: factors determining behavioural tolerance and sensitization. *Psychopharmacol.*, *95*: 534-539.

Martin-Iverson, M.T., Stahl, S.M. and Iversen, S.D. (1987) Factors determining the behavioural consequences of continuous treatment with 4-propyl-9-hydroxynaphthoxazine, a selective dopamine D2 agonist. In F.C. Rose (ed), Parkinson's Disease: clinical and experimental advances. . J. Libbey & Co., London, pp.169-177.

Meller, E., Bohmaker, K., Goldstein, M., and Friedhoff, A.J. (1985) Inactivation of D1 and d2 dopamine receptors by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline in vivo: selective protection by neuroleptics, *J. Pharmacol. Exp. Ther., 233*: 656-662.

Pani, L., Carboni, S., Kusmin, A., Gessa, G.L. and Rossetti, Z.L. (1990) Nimodipine inhibits cocaine-induced dopamine release and motor stimulation, *Eur. J. Pharmacol.*, *176*: 245-246.

Patrick, S.L., Thompson, T.L., Walker, J.M. and Patrick, R.L. (1991) Concomitant sensitization of amphetamine-induced behavioural stimulation and in vivo dopamine release from rat caudate nucleus. *Brain Res. 538*: 343-346.

Pettit, H.O., Pan, H.-T., Parson, L.H. and Justice, J.B. Jr. (1990) Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration, *J. Neurochem.*, *55*: 798-804.

Post, R.M. (1980) Intermittent *versus* continuous stimulation: effect of time interval on the development of sensitization or tolerance, *Life Sci.*, *26:* 1275-1282.

Pucilowski, O. (1992) Psychopharmacological properties of calcium channel inhibitors. *Psychopharmacol.*, *109:*12-29.

Reimer, A.R. and Martin-Iverson, M.T. (1994) Nimodipine and haloperidol attenuate behavioural sensitization to cocaine but only nimodipine blocks the establishment of conditioned locomotion induced by cocaine, *Psychopharmacol. 113:* 404-410.

Reith, M.E.A., Benuck, M. and Lajtha, A. (1987) Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. *J. Pharmacol. Exper. Ther.*, *243:*281-287.

Robinson, T.E. and Becker, J.B. (1982) Behavioural sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro, *Eur. J. Pharmacol.*, *85*: 253-254.

Robinson, T.E. and Becker, J.B. (1986) Enduring changes in brain and behaviour produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis, *Brain Res. Rev., 11*: 157-198.

Robinson, T.E., Becker, J.B. and Presty, S.K. (1982) Long-term facilitation of amphetamine-induced rotational behaviour and striatal dopamine release produced by a single exposure to amphetamine: sex differences, *Brain Res., 253*: 231-241.

Robinson, T.E., Becker, J.B., Young, E.A., Akil, H. and Castaneda, E. (1987) The effects of footshock stress on regional brain dopamine metabolism and pituitary endorphin release in rats previously sensitized to amphetamine, *Neuropharmacology, 26*: 679-691.

Robinson, T.E., Jurson, P.A., Bennett, J.A. and Bentgen, K.M. (1988) Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)amphetamine: a microdialysis study in freely moving rats. *Brain Research, 462*: 211-222.

Saller, C.F., Kreamer, L.D., Adamovage, L.A., and Salama, A.I. (1989) Dopamine receptor occupancy in vivo: Measurement using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), *Life Sci., 45*: 917-929.

Segal, D.S. and Kuczenski, R. (1992a) *In vivo* microdialysis reveals a diminished amphetamine-induced dopamine response corresponding to behavioural sensitization produced by repeated amphetamine pretreatments. *Brain Res. 571*: 330-337.

Segal, D.S. and Kuczenski, R. (1992b) Repeated cocaine administration induces behavioural sensitization and corresponding decreased extracellular dopamine responses in caudate and accumbens. *Brain Res. 577*: 351-355.

Segal, D.S. and Schuckit, M.A. (1983) Animal models of stimulant induced psychosis, In Creese, I. (Ed), *Stimulants: Neurochemical, Behavioural, and Clincal Perspectives*, Raven Press, New York, pp. 131-168.

Silverstone, P.H. and Grahame-Smith, D.G. (1992) A review of the relationship between calcium channels and psychiatric disorders. *J. Psychopharmacol., 6:*462-482.

Stewart, J. and Vezina, P. (1989) Microinjections of SCH 23390 into the ventral tegmental area and substantia nigra pars reticulata attenuate the development of sensitization to the locomotor activating effects of systemic amphetamine, *Brain Res., 495:* 401-406.

Terwilleger, R.Z., Betner-Johnson, D., Servarino, K.A., Crain, S.M. and Nestler, E.J. (1991) A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. *Brain Res., 548:*100-110.

Vezina, P. (1993) Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: an in vivo microdialysis study in the rat. *Brain Res. 605*: 332-337.

Vezina, P. and Stewart, J. (1990) Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. *Brain Res. 516*: 99-106.

CHAPTER 6. CHRONIC COCAINE ADMINISTRATION DOES NOT AFFECT THE OCCUPATION OF 5-HT$_2$ RECEPTORS IN RAT FRONTAL CORTEX

6.1. INTRODUCTION

Studies on the pharmacology of cocaine have largely focused on its effect on brain catecholamines, particularly DA, but there is mounting evidence pointing to a role for 5-HT in mediating cocaine's actions. Cocaine potentiates serotonergic transmission by binding to 5-HT uptake sites (Reith et al., 1983; Reith 1992) to block its uptake into the nerve terminal (Ross and Renyi, 1967), a process found to occur with greater efficacy than uptake inhibition of DA or NA (Ross and Renyi, 1969). The resultant increase in extracellular 5-HT is thought to contribute to cocaine's ability to block 5-HT synthesis (Galloway, 1990). Other acute studies have also demonstrated cocaine's effects on 5-HT function, including heightened sensitivity to 5-HT, as measured via *in vitro* intracellular recordings in the prefrontal cortex after various applications of cocaine (Tanaka and North, 1993). Additional electophysiological investigations have also shown that cocaine inhibits 5-HT unit activity in the DRN, presumably due to autoinhibition caused by the cocaine-induced uptake inhibition (Cunningham and Lakoski, 1988; Pan and Williams, 1989; Pitts and Marwah, 1986, 1987). Administration of procaine or lidocaine did not produce this depressant effect on 5-HT cell firing, thereby ruling out local anesthetic effects of cocaine (Cunningham and Lakoski, 1988; Pitts and Marwah, 1986, 1987). Hyperpolarization of DRN cells by cocaine was, as reported by Pan and Williams (1989), reversed by spiperone, a 5-HT$_2$ antagonist and DA D2 antagonist, thereby adding support to the hypothesis that this effect of cocaine was mediated by its ability to potentiate endogenous 5-HT.

These results suggest that cocaine prolongs the synaptic activity of 5-HT, resulting in autoregulatory inhibition of cell firing in the DRN, the origin of many forebrain 5-HT projection areas. Given the potential for cocaine to influence 5-HT-neurochemistry throughout the brain, it is important to consider its effect when given repeatedly to mimic the pattern of abuse seen in humans (Johanson and Fischman, 1989). Unfortunately, there is limited research on the effects of chronic cocaine on serotonergic function. Kleven et al. (1988) found no changes in 5-HT content or neurotoxicity to 5-HT-containing neurons after repeated doses of cocaine. Similarly, no changes to 5-HT receptor densities have been reported using autoradiography (Zeigler et al., 1991) or *in vitro* binding techniques (Javaid et al., 1993; Johnson et al., 1993). Darmani et al. (1992), reported enhanced 5-HT$_2$ receptor sensitivity to the behavioural effects of the agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) after chronic cocaine, but only with low cocaine doses (0.03-1.25 mg/kg, IP).

The paucity of data available on the effects of chronic cocaine on serotonergic function seems surprising in light of the fact that 5-HT may play an important role in cocaine self-administration behaviour. Carroll et al. (1990) reported that cocaine self-administration is inversely related to 5-HT activity such that blockade of 5-HT uptake by fluoxetine serves to decrease cocaine self-administration by approximately 50%. This finding is somewhat controversial however, since it does not hold true for larger doses of cocaine (Carroll et al., 1990; Porrino et al., 1989). Depletion of forebrain 5-HT by pharmacological lesions with 5,7-dihydroxytryptamine (5,7-DHT) was shown to increase the break-points on progressive ratio schedules of cocaine reinforcement, supporting an inverse relationship between cocaine reward and 5-HT function (Loh and Roberts, 1990). This negative effect of 5-HT on cocaine's reinforcing effects is also supported by the report of Ritz et al. (1987) in which the reinforcing properties of cocaine were positively correlated with its ability to bind to the DA transporter, but revealed a partial negative correlation with 5-HT transporter binding affinity. For cocaine, it appears that its effect on the DA system may be more important for mediating its rewarding aspects, but an interaction between 5-HT and DA systems whereby increased 5-HT tone facilitates DA transmission has also been proposed (Galloway, 1992). The finding that 5-HT increases DA release in the anterior striatum (Galloway, 1992) supports the notion of serotonergic facilitation of DA activity which may result in a decreased requirement for the DA-activating properties of self-administered cocaine. This hypothesis implicates 5-HT in an important interaction with the DA synapse and may extend to other behavioural phenomena associated with repeated cocaine, namely behavioural sensitization.

Recent research investigated the possibility that increased DA release is a substrate for cocaine-induced behavioural sensitization, as measured by receptor occupation in DA terminal fields (Burger and Martin-Iverson, 1994). This study utilized a relatively new tool for measuring receptor function, namely administration of the irreversible receptor antagonist EEDQ. Essentially, this compound alkylates vacant receptors preventing further binding by any endogenous or exogenous ligand (Belleau and Malek, 1968; Belleau et al., 1969). It is a nonspecific antagonist which not only affects the DA receptors but α-adrenergic and a variety of serotonergic receptors as well (Meller et al., 1986). Its receptor nonspecificity makes EEDQ a valuable tool for investigating a number of receptor effects in the same animal. In the present study, the occupation of 5-HT_2 receptors in the frontal cortex was examined in rats given repeated cocaine intermittently to produce sensitization or continuously to produce tolerance. In addition, some animals were pretreated with the L-type Ca^{2+} channel antagonist, nimodipine, based on this compound's ability to block sensitization to cocaine. The purpose was to investigate the possibility that cocaine also induces 5-HT release, which would subsequently be blocked by nimodipine's actions at the cell body. Through these actions in the 5-HT system, cocaine may subsequently influence 5-HT receptor occupation in animals exhibiting behavioural sensitization.

6.2. METHODS

6.2.1 Animals

Male Sprague-Dawley rats (Charles River Canada) weighing 250-350g at the start of the experiment were individually housed on a 12h light-dark cycle (lights on at 0800 h) with food and water available ad libitum. All subjects were weighed every other day throughout the course of the experiment.

6.2.2. Drug Administration Procedure

Part I: After habituation to the colony conditions, 48 rats were assigned to one of 8 drug groups (n=8 in all but vehicle groups which were n=4 for each of the continuous and intermittent drug delivery). Cocaine hydrochloride (BDH, 10 mg/kg/day) or vehicle (double-distilled water) was administered daily via intraperitoneal injection or Alzet^R osmotic minipump (Alza, model 2ML2) for 14 consecutive days. Pump implantation was performed on Day 0, as previously described (see Chapter 3). Some animals received prior injections of nimodipine (10 mg/kg, IP) or its vehicle [polyethylene glycol 400 (100%)] each day, 70 minutes prior to cocaine. On Day 14, each rat received 5mg/kg EEDQ (dissolved in ethanol, propylene glycol and double distilled water, 2:1:2) 30 minutes post-cocaine. Twenty-four hours later, all subjects were decapitated and their brains removed for dissection on ice. Left and right frontal cortex were obtained and frozen at -80 °C for further analysis.

Part II: All procedures were the same as for part I except for the addition of a withdrawal period from cocaine administration. Rats were given cocaine in the same fashion as above for a duration of 10 days. On the 10th day, all minipumps were removed under anesthetic (methoxyflurane) and the rats returned to their home cages. On Day 10 of the withdrawal period, each vehicle treated rat received a challenge dose of cocaine (10 mg/kg, IP) or its vehicle. All other groups received a cocaine challenge. No nimodipine was administered on this drug treatment day. EEDQ was administered as for Part I, with subsequent decapitation and dissection 24 hours later.

6.2.3. 5-HT$_2$ Receptor Binding

Unilateral tissue from rat frontal cortex (left and right sides randomized across drug groups) was homogenized in 4 ml of ice cold TRIS (50 mM, pH 7.4) using the ultra turrax T25 (Janke and Kunkel) set at 22,000 rpm for 3 sec. The homogenate was subsequently centrifuged at 40,000xg for 10 min at 4 °C (Beckman L7-55). The pellet was resuspended, recentrifuged and resuspended in TRIS to a final volume of 6 ml. Tubes were set up in duplicate containing TRIS, ^{3}[H] ketanserin (hot ligand), mianserin (10 μm, cold competitor) and tissue to a final volume of 1 ml. Ligand concentrations were 4.0, 2.0, 1.0, 0.5, 0.25 and 0.125 nM. After incubation at 37 °C for 15 min, samples were subjected to filtration through a Brandel cell harvester with Whatman filters pre-soaked for 3 h in 0.1% polyethyleneimine in H_2O. The filters were rinsed twice with TRIS and placed in vials containing 5 ml scintillation cocktail. After 12 h at room temperature, the amount of radioactivity in each vial was measured using liquid scintillation counting (see Chapter 2). The resultant counts were analyzed using EBDA/LIGAND. Protein content was determined by the method of Lowry et al. (1951).

6.2.4. Statistics

Receptor density (Bmax) and affinity (K_d) values were subjected to ANOVA with planned comparisons using multiple F tests, where appropriate. Factors considered included nimodipine (0 or 10 mg/kg), cocaine (0 or 10 mg/kg) and treatment (continuous or intermittent). For Part II, the cocaine and treatment factors were combined (0, intermittent or continuous) and the data expressed as percent of vehicle animals with a vehicle challenge.

6.3. RESULTS

After 14 days of cocaine administration (Part I), the density of 5-HT_2 receptors protected from EEDQ inactivation in rat frontal cortex was increased to 165% of vehicle controls in those animals receiving cocaine via intermittent injections (Fig. 6-1). This is compared to a level of 123% of control for the continuous administration group. This trend toward higher occupation of 5-HT_2 receptors with cocaine delivered in an intermittent fashion was not significant after ANOVA. Similarly, nimodipine pretreatment did not significantly alter 5-HT_2 receptor levels in this group of rats. With respect to K_d, as shown in Table 6-1, nimodipine treatment significantly decreased receptor affinity after 14 days of

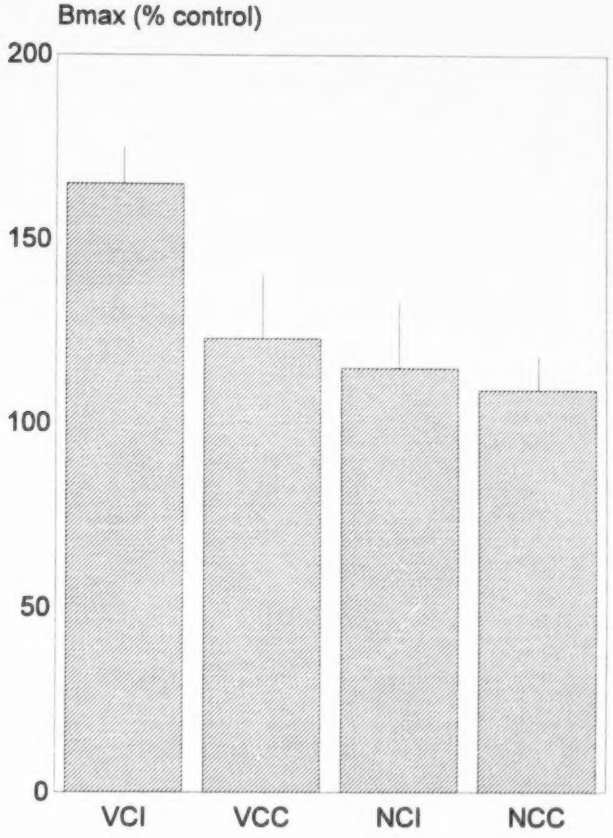

Fig. 6-1. Mean 5-HT$_2$ receptor Bmax values (± SEM) in rat frontal cortex represented as percent of vehicle control after 14 days of chronic cocaine administered either intermittently (CI) or continuously (CC). Some animals also received prior administration of nimodipine (N) or its vehicle (V). Control Bmax=256.06 ±50.18 (intermittent), 312.0 ±68.25 (continuous).

Table 6-1. $5HT_2$ Receptor K_d values for Part I. * p<.05 significance from vehicle control.

TREATMENT	K_d (nM) ± SE
Vehicle - intermittent	1.039 ± .252
- continuous	0.907 .128
Cocaine - intermittent	0.917 .133
- continuous	1.087 .100
Nimodipine + vehicle - intermittent	1.126 .296
- continuous	1.681 .282*
Nimodipine + cocaine - intermittent	1.414 .205
- continuous	1.330 .155

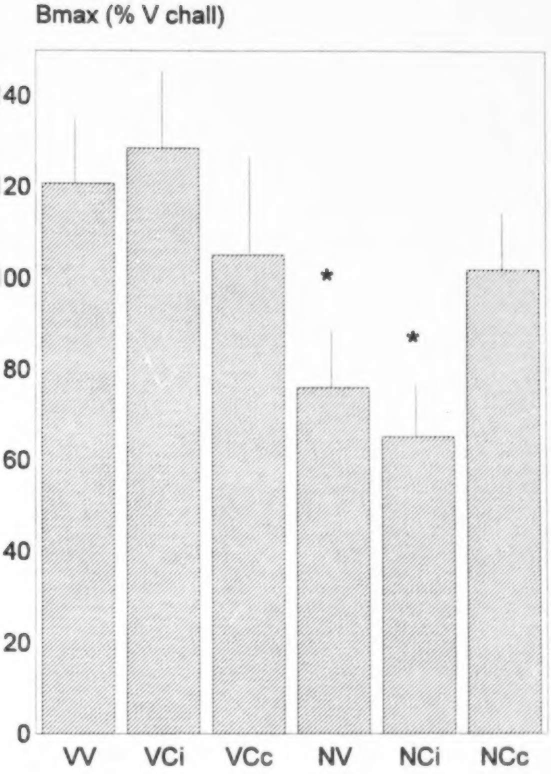

Fig. 6-2. 5-HT$_2$ receptor occupation (mean ± SEM) in rat frontal cortex after chronic cocaine administered continuously or intermittently for 10 days followed by a 10 day withdrawal period and subsequent cocaine challenge (Chall). Bmax values are represented as percent of vehicle animals receiving a vehicle challenge on the test day. Control Bmax= 361.30 ± 72.45. Drug groups include: Vehicle animals with cocaine challenge (VV), cocaine given intermittently (VCi), continuous cocaine (VCc), nimodipine alone (NV), nimodipine and cocaine intermittently (NCi) and nimodipine with continuous cocaine (NCc). * $p<.05$ significantly different from VV.

treatment ($F(1,39)=8.66$, $p<.01$).

In Part II, 10 days of cocaine treatment and a single challenge after a withdrawal period did not significantly affect $5HT_2$ receptor Bmax. In Fig. 6-2, vehicle animals treated with cocaine for the first time on the challenge day had a receptor occupation level of 121% of vehicle animals given a vehicle challenge. Prior treatment with cocaine (intermittently) only increased this level by an additional 8% of control. Unlike the findings from Part I of this study, nimodipine pre-treatment had a significant effect on both Bmax and K_d (Table 6-2). Prior administration of nimodipine during the chronic phase of this experiment served to reduce the level of protection from EEDQ when given alone or in combination with intermittent, but not continuous cocaine.

6.4. DISCUSSION

The administration of cocaine has been associated with the development of behavioural sensitization if given intermittently or tolerance via continuous treatment regimens (Burger and Martin-Iverson, 1994; Post, 1980). These different behavioural effects have been correlated with changes in the DA system in an attempt to understand the neural substrates of sensitization and tolerance. Recently, it has been shown that intermittent cocaine delivery producing locomotor sensitization is accompanied by an increase in the occupation of D1 and D2 receptors, measured as protection from EEDQ denaturation, in the striatum and NAS (see Chapter 5). No changes were found after continuous administration associated with behavioural tolerance. Interestingly, pharmacological blockade of cocaine-stimulated DA release by nimodipine (Pani et al., 1990) was also able to block this increased

Table 6-2. 5-HT_2 receptor affinity values for Part II. * $p<.05$ significance from vehicle control.

TREATMENT	K_d (nM) ± SE	
Cocaine - 0 (vehicle)	1.031 ±	.195
- intermittent	1.020	.137
- continuous	0.899	.135
Nimodipine + cocaine - 0 (vehicle)	1.751	.298*
- intermittent	1.266	.187
- continuous	1.286	.099

DA receptor occupation. These results suggest increased stimulated DA release as a mechanism associated with behavioural sensitization. However, when a period of withdrawal from cocaine is included in the drug administration paradigm, followed by an acute cocaine challenge, these findings no longer hold true. In fact, receptor occupancy in the striatum is not correlated with behavioural sensitization or tolerance since intermittent cocaine history does not significantly increase D1 or D2 receptor occupation above that of vehicle animals given acute cocaine challenge (see Chapter 5). It therefore appears that the treatment regimens produce different effects on receptor occupancy because of different levels of cocaine in the brain at the time of EEDQ administration. Recent research has suggested a role for 5-HT in mediating the reinforcing effects of cocaine and, given the potent effects of cocaine at the 5-HT synapse, implicate this neurotransmitter system in a wide variety of cocaine's effects. Furthermore, recent microdialysis experiments have shown augmented extracellular 5-HT levels in the NAS, VTA and DRN in response to a cocaine challenge in cocaine-sensitized animals (Parsons and Justice, 1993). Therefore, use of EEDQ to measure receptor occupation has been extended to include the frontal brain regions in the present study in an attempt to investigate the role of 5-HT in cocaine-induced behavioural sensitization both before and after a drug withdrawal period.

Essentially, the number of 5-HT_2 receptors protected from EEDQ inactivation after chronic cocaine was increased in the frontal cortex for the intermittent delivery group, regardless of a drug holiday, but not significantly so. This finding is in accordance with the few receptor binding studies performed to date which have examined changes in 5-HT receptor Bmax after chronic cocaine. Johnson et al. (1993) administered cocaine twice daily

(20 mg/kg) for two weeks only to report no changes in 5-HT_2 density in the hippocampus, caudate-putamen or frontal cortex. Similarly, cocaine given chronically (intermittently) at 10 mg/kg increased stereotypy ratings but failed to alter 5-HT_2 receptor Bmax significantly in rat cortex. Autoradiographic analysis of chronic cocaine's effects on 5-HT binding in rat brain also found no changes with either intermittent or continuous (via cocaine pellet) delivery for 5 days followed by a 30-day withdrawal period (Zeigler et al., 1991). Based on these data, it would appear that cocaine's ability to block 5-HT uptake is not sufficient to alter receptor number or, as shown in the present study, significantly increase receptor occupation, when delivered in intermittent or continuous treatment regimens.

The reports of cocaine's inability to significantly alter postsynaptic receptor events even though uptake inhibition is achieved warrants an evaluation of presynaptic events in the DRN. In the DRN, autoinhibitory processes, occur at 5-HT cells via connections from other 5-HT neurons. When cocaine blocks 5-HT uptake processes more of this neurotransmitter is available to interact with autoreceptors on neighboring dendrites, thereby dampening 5-HT cell activity (Cunningham and Lakoski, 1988; Pan and Williams, 1989; Pitts and Marwah, 1986, 1987). After chronic exposure to cocaine, however, no reported changes in 5-HT DRN physiology have been reported with the same cocaine dose and intermittent injection treatment regimen (10 mg/kg for 10 days) used in the present study (Cunningham et al, 1987). If this dose was tripled and given twice daily for 7 days, subsequent cocaine perfusion on 5-HT DRN neurons revealed tolerance or reduced sensitivity (Black and Lakoski, 1990). Subsequent analysis of 5-HT_{1A} receptor Bmax and K_d levels showed no changes with this chronic treatment regimen. These findings suggest that chronic exposure to cocaine (at very high doses) reduces sensitivity of the 5-HT DRN neurons but not via presynaptic changes in autoreceptor density (Lakoski et al., 1992). At the dose level employed in the present investigation, cocaine would not be expected to affect 5-HT cell activity significantly enough to alter post-synaptic events (via this mechanism) after prolonged exposure.

The apparent lack of evidence for significant changes in 5-HT neurochemistry following chronic cocaine administration at doses previously associated with behavioural sensitization is puzzling in light of the finding that cocaine blocks uptake of 5-HT with a greater affinity than that for DA or NA (Ross and Renyi, 1969). Adding to the confusion is the present finding of a nimodipine effect on blocking cocaine-induced 5-HT_2 receptor occupation when given alone for 10 days or concurrently with intermittent injections of cocaine. Considering that nimodipine has been shown to block the impulse-dependent release of DA induced by cocaine (Pani et al., 1990) in conjunction with blocking increased DA receptor occupation in behaviourally sensitized rats (Burger and Martin-Iverson, 1994) it is possible that cocaine also affects 5-HT release. Wolf and Kuhn (unpublished observations) report that cocaine (given *in vivo*) can increase the stimulated release of 5-HT from synaptosomes (cited in Wolf and Kuhn, 1991). It is also feasible that cocaine could regulate 5-HT release via its action at the 5-HT uptake site since many autoreceptor agonists that suppress release also bind to the 5-HT transporter to inhibit uptake (Wolf and Kuhn, 1989). The most convincing research to date on cocaine's effects on 5-HT release comes from a recent report of increased extracellular levels of 5-HT in mesolimbic regions after repeated cocaine administration (Parsons and Justice, 1993). This research did not measure

5-HT levels after a withdrawal period so it is difficult to assess if these changes are permanent and mediate the long-term behavioural sensitization seen with repeated cocaine. Unfortunately, more research is needed on the effects of cocaine and 5-HT release before any definitive conclusions on this matter can be reached. As far as the ability of nimodipine to block the effect of cocaine (both acute and chronic treatment) on 5-HT receptor occupation is concerned, it does not appear that nimodipine is able to affect the 5-HT system directly. This class of L-type calcium channel antagonists has not been associated with changes in basal 5-HT release (Schlicker et al., 1991). Similarly, no changes in ^{3}[H]ketanserin binding to 5-HT$_2$ receptors in rat cerebral cortex have been reported (Adachi and Shoji, 1986). These studies support the notion that nimodipine is producing the present results via its ability to block cocaine-induced 5-HT release. In other words, cocaine may be inducing 5-HT release in the same process as for DA, since it is blocked by the same pharmacological agent. The finding that nimodipine influences 5-HT$_2$ receptor affinity in the present study could be due to the fact that it was chronically administered, unlike in the study of Adachi and Shoji (1986), or to differences in the actions of verapamil (another L-type Ca^{2+} channel antagonist) used by these authors, and nimodipine.

The present experiment was designed to explore the role of 5-HT in cocaine-induced behavioural sensitization. The lack of significant changes in receptor occupation suggests that cocaine's ability to block uptake of 5-HT to produce both pre- and postsynaptic effects with acute doses does not significantly alter 5-HT neurochemistry after chronic administration. However, the importance of 5-HT in mediating cocaine's effects on the brain and behaviour has only just begun to be explored. The present study suggests that cocaine may influence 5-HT release to increase postsynaptic receptor occupation, a process blocked by nimodipine. It should be noted, however, that only receptors in the frontal cortex were examined. 5-HT_2 receptor occupation in other terminal fields more specifically involved in behavioural sensitization (i.e. NAS and striatum) may elucidate the role of 5-HT, and its possible interaction with DA, in cocaine-induced behavioural sensitization.

6.5. REFERENCES

Adachi, H. and Shoji, T. (1986) Characteristics of the inhibition of ligand binding to serotonin receptors in rat brain membranes by verapamil. *Jpn. J. Pharmacol., 41:* 431-435.

Belleau, B., DiTullio V., and Godin, D. (1969) The mechanism of irreversible adrenergic blockade by N-carbethoxydihyroquinolines--model studies with typical serine hydrolases. *Biochem. Pharmacol., 18:* 1039-1044.

Belleau, B. and Malek, G. (1968) A new convenient reagent for peptide syntheses. *J. Am. Chem. Soc., 90:* 1651-1652.

Black, E.W. and Lakoski, J.M. (1990) In vitro electrophysiology of dorsal raphe′ serotonergic neurons in subchronic cocaine treated rats: development of tolerance to acute cocaine administration. *Mol. Cell. Neurosci., 1:* 84.

Burger, L.Y. and Martin-Iverson, M.T. (1994) Increased occupation of D1 and D2 DA receptors accompanies cocaine-induced behavioural sensitization. *Brain Res., 639:* 228-232.

Carroll, M.E., Lac, S.T., Asencio, M. and Kragh, R. (1990) Fluoxetine reduces intravenous cocaine self-administration in rats. *Pharmacol. Biochem. Behav., 35:* 237-244.

Cunningham, K.A., Asprodini, E.K., Bernau, N.A., Richard, C.A. and Lakoski, J.M. (1987) Enhanced inhibitory responses of serotonin neurons in the dorsal raphe nucleus (DRN) after repeated cocaine exposure. *Soc. Neurosci. Abstr., 13, Part 3:* 1651.

Cunningham, K.A. and Lakoski, J.M. (1988) Electrophysiological effects of cocaine and procaine on dorsal raphe′ serotonin neurons. *Eur. J. Pharmacol., 148:* 457-462.

Darmani, N.A., Martin, B.R. and Glennon, R.A. (1992) Repeated administration of low doses of cocaine enhances the sensitivity of 5-HT_2 receptor function. *Pharmacol. Biochem. Behav., 41:* 519-527.

Galloway, M.P. (1990) Regulation of dopamine and serotonin synthesis by acute administration of cocaine. *Synapse, 6:* 63-72.

Galloway, M.P. (1992) Neuropharmacology of cocaine: effects on dopamine and serotonin systems. In J.M. Lakoski, M.P. Galloway and F.J. White (Eds.), *Cocaine: Pharmacology, physiology, and clinical strategies,* CRC Press, Boca Raton, FL, pp. 163-189.

Javaid, J.I., Sahni, S.K., Pandey, S.C. and Davis, J.M. (1993) Repeated cocaine administration does not affect 5-HT receptors subtypes (5-HT_{1A}, 5-HT_2) in several rat brain regions. *Eur. J. Pharmacol. 238:* 425-429.

Johanson, C.-E. and Fischman, M.W. (1989) The pharmacology of cocaine related to its abuse. *Pharmacol. Rev., 41:* 3-52.

Johnson, R.G., Fiorella, D. and Rabin, R.A. (1993) Effects of chronic cocaine administration on the serotonergic system in the rat brain. *Pharmacol. Biochem. Behav., 46:* 289-293.

Kleven, M.S., Woolverton, W.L., and Seiden, L.S. (1988) Lack of long-term monoamine depletions following repeated or continuous exposure to cocaine. *Brain Res. Bull., 21:* 233-237.

Lakoski, J.M., Black, E.W. and Moday, H.J. (1992) Electrophysiologic effects of cocaine on serotonin neuronal systems. In J.M. Lakoski, M.P. Galloway and F.J. White (Eds.), *Cocaine: Pharmacology, physiology, and clinical strategies*, CRC Press, Boca Raton, FL, pp. 295-311.

Loh, E.A. and Roberts, D.C.S. (1990) Break-points on a progressive ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. *Psychopharmacol., 101:* 262-266.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurements with the Folin phenol reagent. *J. Biol.Chem., 193:*265-275.

Meller, E., Goldstein, M. Friedhoff, A.J. and Schweitzer, J.W. (1986) N-Ethoxycarbonyl -2-ethoxy-1,2-dihydroquinoline (EEDQ): A new tool to probe CNS receptor function. In M. Goldstein, K. Fuxe, I. Tabachnick (Eds), *Central D1 dopamine receptors*, Plenum Press, New York, pp. 121-136.

Pani, L ., Carboni, S., Kusmin, A., Gessa, G.L. and Rossetti, Z.L. (1990) Nimodipine inhibits cocaine-induced dopamine release and motor stimulation. *Eur. J. Pharmacol., 176:* 245-246.

Pan, Z.Z. and Williams, J.T. (1989) Differential actions of cocaine and amphetamine on dorsal raphe neurons *in vitro*. *J. Pharmacol. Exp. Ther., 251:* 56-62.

Parsons, L.H. and Justice, J.B. Jr. (1993) Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area and dorsal raphe´ nucleus following repeated cocaine admininstration. *J. Neurochem.,61:*1611-1619.

Pitts, D.K. and Marwah, J. (1986) Electrophysiological effects of cocaine on central monoaminergic neurons. *Eur. J. Pharmacol., 131:* 95-98.

Pitts, D.K. and Marwah, J. (1987) Cocaine modulation of central monoaminergic neurotransmission. *Pharmacol. Biochem. Behav., 26:* 453-461.

Porrino, L.J., Ritz, M.C., Goodman, N.L., Sharpe, L.G., Kuhar, M.J. and Goldberg, S.R. (1989) Differential effects of the pharmacological manipulation of serotonin systems on cocaine and amphetamine self-administration in rats. *Life Sci., 45:* 1529-1535.

Post, R.M. (1980) Intermittent versus continuous stimulation: effect of time interval on the development of sensitization or tolerance. *Life Sci., 26:* 1275-1282.

Ritz, M.C., Lamb, R.J., Goldberg, S.R. and Kuhar, M.J. (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 237:* 1219-1224.

Reith, M.E.A., Sershen, H., Allen, D.L. and Lajtha, A. (1983) A portion of ^{3}[H]cocaine binding in brain is associated with serotonergic neurons. *Mol. Pharmacol., 23:* 600-606.

Reith, M.E.A. (1992) Central and peripheral cocaine binding sites. In J.M. Lakoski, M.P. Galloway and F.J. White (Eds.), *Cocaine: Pharmacology, physiology and clinical strategies,* CRC Press, Boca Raton, FL, pp. 203-227.

Ross, S.B. and Renyi, A.L. (1967) Inhibition of the uptake of tritiated catecholamines sby antidepressant and related agents. *Eur. J. Pharmacol., 2:* 181-186.

Ross, S.B. and Renyi, A.L. (1969) Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. *Eur. J. Pharmacol., 7:* 270-277.

Schlicker, E., Glaser, T., Lummen, G., Neise, A. and Gothert, M. (1991) Serotonin and histamine receptor-mediated inhibition of serotonin and noradrenaline release in rat brain cortex under nimodipine treatment. *Neurochem. Int., 19:* 437-444.

Tanaka, E. and North, R.A. (1993) Cocaine enhancement of the action of 5-hydroxytryptamine in rat cingulate cortex in vitro. *Neurosci. Lett., 163:* 50-52.

Wolf, W.A. and Kuhn, D.M. (1989) Inhibition of 5-HT uptake by the 5-HT1B agonist TFMPP. *Soc. Neurosci., 15:* 224.

Wolf, W.A. and Kuhn, D.M. (1991) Cocaine and serotonin neurochemistry. *Neurochem. Int., 18:* 33-38.

Zeigler, S., Lipton, J., Toga, A. and Ellison, G. (1991) Continuous cocaine administration produces persisting changes in brain neurochemistry and behaviour. *Brain Res., 552:* 27-35.

CHAPTER 7. GENERAL DISCUSSION

The psychomotor stimulant cocaine is associated with behavioural tolerance when administered continuously or sensitization if given intermittently. This dissociation of behavioural effects depending on the regimen of adminstration has been linked to long-term changes in dopaminergic neurotransmission, an area of research that is still controversial. The rationale for behavioural sensitization as an animal model of stimulant-induced psychosis, a disorder generally associated with chronic stimulant administration that is virtually indistinguishable from paranoid schizophrenia (Angrist, 1983; Segal and Schuckit, 1983), is based on the premise that long term neurochemical changes associated with animal behavioural homologues of chronic stimulant-induced psychoses will be similar to neurochemical substrates of human stimulant psychoses. In this thesis are described the effects of chronic cocaine administration on DA and 5-HT receptor function in an attempt to further elucidate the neural mechanisms of sensitization to cocaine. This objective is achieved through the use of a relatively new tool, receptor alkylation, which enables the investigator to indirectly measure the amount of DA (and 5-HT) bound to receptors by assessing the density of occupied receptor sites with standard radiolabelled receptor assays. These data, in addition to the extensive analysis of the structure of behaviour, provide novel and original information about the effects of chronic cocaine on brain and behaviour.

The rationale behind the use of EEDQ as a tool for understanding receptor function is based on its irreversible actions at a number of receptor sites that are not protected by agonists or antagonists. As described in Chapter 2, this compound denatures vacant receptors, allowing for measurement of sites occupied by endogenous or exogenous ligands (Belleau et al., 1969). EEDQ denatures 50% of DA receptors significantly with doses as low as 5 mg/kg, without further altering the functioning DA system. Cocaine, administered prior to EEDQ, protects both D1 and D2 receptors from EEDQ denaturation. In other words, acute doses of cocaine increase the amount of DA available for binding to receptors. EEDQ therefore allows for an indirect measurement of extracellular neurotransmitter levels. From a practical standpoint, EEDQ adminstration also offers several advantages over other *in vivo* techniques like microdialysis and PET scans with *in vivo* radioligand adminstration, which are more invasive and more expensive. The nonselectivity of EEDQ is also advantageous in that it allows for an assessment of the occupation of a number of different receptor subtypes. This method is not without its problems, however, since there is some confusion as to its exact mechanism at the receptor site based on findings that support the idea that EEDQ only acts at the external surface (Meller et al., 1986). However, the carboxy terminal target for EEDQ inactivation is located intracellularly for monoamine receptors, probably on transmembrane domain 2 or 3 (Gozlan, et al., 1994). It may be possible that EEDQ gains intraneuronal access to denature receptors without further affecting the intracellular mediators that regulate cAMP. Given the highly reactive nature of this compound however, this is unlikely. The metabolism of EEDQ has not been fully investigated either and, since EEDQ is peripherally administered in the present experiments, metabolism may significantly influence the amount of EEDQ that reaches the brain. Its actions include inhibition of cholinesterases in the body (Belleau et al., 1969) which may lead to inhibition of its own metabolism. Finally, a complete time course for EEDQ effects has not been examined adequately in the literature. EEDQ inactivation of DA D1 receptors has shown no significant differences in receptor degradation at 4 and 24 hours post-injection (Nowak, 1990). The study of Fuxe et al. (1987), however, reports some D1 receptor recovery between these two time points and calculates an overall half-life of 34.0 h in the rat striatum. Receptor reappearance is therefore fairly rapid after EEDQ administration, depending on the receptor subtype (Fuxe et al., 1987). EEDQ would not appear to have a prolonged duration of action based on these findings since further degradation is no longer occuring after the 4 h mark. In addiiton, the effect of EEDQ on behaviour (i.e. CNS depressant actions and catalepsy) has been shown to occur rapidly following administration (Belleau et al., 1968). For the present experiments, given the short duration of action of cocaine, this rapid effect of EEDQ does not pose a problem, but more research on the use of EEDQ would provide a better understanding of the mechanisms at work. For now, the use of this tool provides a new approach in the investigation of neural substrates of sensitization and tolerance to psychomotor stimulants.

Continuous infusions of psychomotor stimulants have produced differential results depending on the circadian cycle. The DA agonist amphetamine (Martin-Iverson and Iversen, 1989) and PHNO (Martin-Iverson et al., 1988b), a D2 agonist, delivered continuously for 2 weeks produced nocturnal sensitization and daytime tolerance to the locomotor stimulating effects of these drugs. These effects have been proposed to be mediated by D1 receptor supersensitivity induced by circadian fluctuations in DA release.

It was hypothesized that decreased release at D1 sites during the day produces a loss of activation and a subsequent increase in density. At night, DA release is increased and, accompanied by increased D1 receptors, produces behavioural sensitization. Further support for this hypothesis comes from the finding that daytime tolerance can be reversed by co-administration of a D1 agonist and the D1 antagonist SCH23390 blocks the initial locomotor stimulation effect of PHNO (Martin-Iverson, 1988a). Chronic administration of cocaine via continuous infusion increased the occupation of D1 receptors, depending on the circadian cycle, but did not significantly alter D2 receptor occupation. The day/night difference in receptor occupation at D1 sites was reversed by co-administration of SCH 23390. These data add support to the notion of a phasic role for D1 receptors in response to cocaine.

A detailed analysis of behaviour over 2 weeks of continuous cocaine treatment was originally undertaken in order to examine the behavioural differences associated with tolerance and sensitization. This experiment included more than the simple locomotor activity counts described previously with chronic amphetamine and PHNO administration. Time sampling with video equipment provided information on the frequencies, durations and transitions between 28 separate behaviours for the first and last day of cocaine treatment. This extensive behavioural analysis was examined within the framework of existing behavioural theories regarding the effects of other psychomotor stimulants, particularly amphetamine. Tolerance to cocaine's locomotor stimulating effects was exhibited regardless of the circadian cycle, measured with photocell activity counts. This finding dissociates cocaine from the circadian effects reported with amphetamine and

PHNO. More detailed analysis of rat exploratory activity confirmed this significant pattern of tolerance with greater initial stimulant effects at night, compared to the day. Further analysis of the structure of behaviour, as documented by sequence transitions, reveals that the initial stimulant effects of cocaine serve only to enhance the frequency of behaviour patterns without altering the underlying structure. These results have negative implications for the Lyon and Robbins (1975) hypothesis of stimulant effects on behaviour. According to this theory, increasing doses of amphetamine result in a narrowing of the response repertoire to more simple patterns of behaviour. This is proposed to be due in part to competition between response sequences (Robbins and Sahakian, 1983). Overall, cocaine administration resulted in competition between quiet behaviours like sleeping and lying down and exploratory activity. With increasing doses of cocaine, however, increased behavioural competition was not evident between locomotion, a behaviour more likely to be associated with the lower doses of cocaine, and oral stereotypy, seen with higher doses. Enhanced switching between responses or abortive response sequences, another corollary of the Lyon and Robbins hypothesis, was also not supported with cocaine administration. Instead, increasing doses of cocaine simply increased the overall frequency of these behaviours through sequences seen in vehicle-treated animals. These findings either further separate cocaine from the effects of other psychomotor stimulants or suggest that new theories of behavioural stimulant action need to be developed to include cocaine. More recently, research has shown that response switching is not necessarily enhanced by amphetamine (Martin-Iverson and DiLullo, unpublished observations; Ziriax et al., 1993). Perhaps the behavioural effects of psychomotor stimulant adminstration are dependent on the features of the test environment and the initial response to the cues therein. Damianopoulos and Carey (1993) propose that sensitization to cocaine is not necessarily expressed as hyperlocomotion, depending on the animal's initial contact with the test cage and its response to the sensorimotor information obtained from that experience. This behavioural reorganization account of repeated exposure to stimulants involves changes in behavioural patterns that often include more features of the test environment with each successive exposure (Damianopoulos and Carey, 1992). Nocturnal sensitization with amphetamine, but not cocaine, may be mediated by their different effects on DA cell activity or release which interacts with fluctuations in DA function with the circadian cycle. These differential effects on the DA system at night compared to the day may lead to different adaptive motor responses to the environmentally relevant stimuli. With repeated exposure to this environment in the drug state, these responses are enhanced and become sensitized as part of a behavioural reorganization effect. This theory resolves to explain why locomotor sensitization is not always a robust effect with repeated stimulant administration. With respect to tolerance, however, it would seem that the initial drug effects that stimulate behaviour are "reorganized" to vehicle levels over successive days of exposure. Perhaps this is due to tolerance in D2 receptor function, which may be more homeostatically controlled, as shown in Chapter 3. These data not only highlight the importance of examining the effects of the circadian cycle on brain and behaviour, but also demonstrate the differences among the psychomotor stimulants and their effects on behaviour.

The lack of behavioural sensitization to continuously administered cocaine, even during the dark portion of the day/night cycle, is different from the literature regarding continuous infusion of amphetamine or PHNO, but other studies examining the effects of cocaine without consideration of the circadian cycle, lend support to the conclusion of exclusive development of tolerance to the locomotor- and stereotypy-inducing properties of cocaine (King et al., 1992, 1993; Reith et al., 1987). This is in contrast to the effects of psychomotor stimulants with intermittent daily injections, namely the development of a heightened behavioural response over successive treatments. The search for a mechanism for sensitization to psychomotor stimulants, including cocaine, has employed a variety of investigative techniques in an attempt to probe the DA system. In Chapter 5, EEDQ administration allowed for a new approach to test the hypothesis of increased interactions of DA with its receptors in the striatum and NAS as a possible mechanism for cocaine-induced sensitization. This investigation lead to the dissociation of the effects of continous vs. intermittent cocaine on behaviour from changes in DA release at both D1 and D2 sites. The development of sensitization to cocaine is not correlated with increased occupation of either DA receptor subtype in the striatum. It remains possible that other terminal field regions are more important than the striatum. The NAS has been implicated in behavioural sensitization, with similar findings to those seen in the striatum. However, due to technical problems, no data are available regarding DA receptor occupation in the NAS in this thesis. The finding that the PFC will support intracranial self-administration of cocaine but not amphetamine (Dworkin and Smith, 1992) points to this terminal field as a possible target for changes in DA receptor function with chronic administration. In fact, it has recently been shown that behavioural sensitization to cocaine is associated with decreased DA transmission in the PFC (Sorg and Kalivas, 1993). It has been proposed that this effect may be due to changes in impulse-dependent release of DA since an amphetamine challenge, which affects impulse-independent cytoplasmic pools of DA, increases extracellular DA in the PFC in cocaine-sensitized animals.

The nonselectivity of EEDQ denaturation is advantageous in that a number of receptor systems can be examined in the same animal. Since no significant changes in DA receptor occupation were associated with chronic cocaine at intermediate doses, the search for a mechanism of sensitization was extended to an examination of 5-HT2 receptor occupation in the frontal cortex. Given the potent uptake blocking efficacy of cocaine at the 5-HT synapse, there is a potential for significant effects of cocaine on serotonergic neurotransmission. Recent research studies have shown increases in extracellular 5-HT levels in cocaine-sensitized animals (Parsons and Justice, 1993). In addition, a role for the PFC in mediating cocaine's effects on behaviour has also been suggested by reports of changes in DA neurotransmission after repeated cocaine treatment (Sorg and Kalivas, 1993). However, as with previous reports on the density of 5-HT2 receptors in various limbic areas after cocaine, no changes in receptor occupancy were found in the frontal cortex in this investigation. If the 5-HT system is involved in cocaine-induced sensitization, the effects may be mediated by other receptor types or involve cell body regions in the DRN. Investigations into the interaction between 5-HT and DA may also prove to be important given that stimulation of 5-HT transmission in the VTA or NAS increases DA release in the NAS (Chen et al., 1991).

The pharmacological blockade of cocaine-induced sensitization by the L-type calcium channel antagonist nimodipine was accompanied by a similar blockade in both DA and 5-HT receptor occupation. However, a dissociation between this blockade of receptor occupation and behaviour was observed after a period of drug withdrawal. These results add further support to the finding that increased interaction of DA with D1 or D2 receptors or 5-HT with 5-HT_2 receptors are not related to the development of behavioural sensitization. The efficacy of nimodipine in reducing the occupation of these receptor subtypes with chronic cocaine does, however, provide evidence that cocaine given intermittently enhances the impulse-dependent release of DA and 5-HT.

7.1. CONCLUSIONS

The conclusions drawn from the investigations in this thesis indicate that cocaine has a different behavioural profile than other psychomotor stimulant drugs, namely amphetamine and PHNO, when given continuously and examined over the entire circadian cycle. The tolerance associated with this continuous drug delivery is for exploratory behaviours as a whole and not simply locomotor activity measured separately by photocell beam interruptions. Extensive behavioural analysis revealed that existing theories of stimulant effects on behaviour do not adequately explain the effects of cocaine administration. An examination of DA receptor occupation with continuously adminstered cocaine points to a dopaminergic mechanism of differential DA release at D1, but not D2, sites in mediating the effects of chronic cocaine. Further analysis of this relationship between DA release measured as the occupation of DA receptors and the behavioural effects of cocaine failed to support this notion of a D1 receptor-mediated mechanism, at least in the striatum. The usefulness of EEDQ as a tool for examining receptor function was further supported in its ability to denature receptors in another neurotransmitter system: 5-HT. Although no effects of cocaine treatment were found in the frontal cortex, this research opens the door to an examination of other regions and receptors in the brain.

7.2. FUTURE DIRECTIONS

The research in this thesis has tested the use of a receptor alkylating agent as a probe for CNS function. EEDQ can be used not only with receptor binding via filtration but also by autoradiography. In addition, EEDQ has been injected directly into specific brain regions (Cameron and Crocker, 1989). These authors were able to create pharmacological lesions of specific areas of the striatum and NAS in an attempt to localize the stereotyped head-down sniffing response to apomorphine. This method therefore provides an opportunity for an analysis of receptor occupation in the smaller cell body regions of the DA and 5-HT systems where, as suggested by a number of electrophysiological studies, cocaine has profound effects on cell function (Henry and White, 1992). Given also the evidence that sensitization to psychomotor stimulants may well be mediated by D1 receptors on DA cell bodies in the VTA and SN (Stewart and Vezina, 1989; Vezina, 1993; Vezina and Stewart, 1990), the use of EEDQ as a measure of synaptic DA in these regions would add strength to these proposed presynaptic mechanisms. More work could also follow up on the possibility that cocaine's ability to affect impulse activity in monoamine systems may be important for its behavioural effects.

Administration to produce tolerance to cocaine should be investigated as to the efffect of cocaine challenge on DA cell firing. In addition, the interaction of 5-HT and DA and the neural connections between these systems should be explored further. Finally, an investigation of other receptor subtypes in the DA and 5-HT systems may provide valuable information about the neural circuitry of behavioural sensitization.

7.3. REFERENCES

Angrist, B. (1983) Psychoses induced by central nervous system stimulants and related drugs, In Creese, I. (Ed), *Stimulants: neurochemical, behavioural, and clinical perspectives*, Raven Press, New York, pp. 1-30.

Belleau, B., DiTullio V. and Godin, D. (1969) The mechanism of irreversible adrenergic blockade by N-carbethoxydihydroquinolines--model studies with typical serine hydrolases. *Biochem. Pharmacol., 18:* 1039-1044.

Belleau, B., Martel, R., Lacasse G., Menard, M., Weinberg, N.L. and Perron, Y.G. (1968) N-carboxylic acid esters of 1,2- and 1,4-dihydroquinolines. A new class of irreversible inactivators of the catecholamine α receptors and potent central nervous system depressants. *J. Am. Chem. Soc., 90:* 823-824.

Cameron, D.L. and Crocker, A.D. (1989) Localization of striatal dopamine receptor function by central injection of an irreversible receptor antagonist. *Neurosci., 32:*769-778.

Chen, J., Van Praag, H.M. and Gardner, E.L. (1991) Activation of 5-HT3 receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. *Brain Res., 543:*354-357.

Damianopoulos, E.N. and Carey, R.J. (1993) Apomorphine sensitization effects: Evidence for environmentally contingent behavioural reorganization processes. *Pharmacol. Biochem. Behav., 45:*655-663.

Damianopoulos, E.N. and Carey, R.J. (1992) Conditioning, habituation and behavioral reorganization factors in chronic cocaine effects. *Behav. Brain. Res. 49:*149-157.

Dworkin, S.I. and Smith, J.E. (1992) Cortical regulation of self-administration. *Annals NY Acad. Sci., 654:*274-281.

Fuxe, K., Agnati, L.F., Pich, E.M., Meller, E. and Goldstein, M., (1987) Evidence for fast receptor turnover of D1 dopamine receptors in various forebrain regions of the rat. *Neurosci. Lett., 81:* 183-187.

Gozlan, H., Laporte, A.-M., Thibault, S., Schechter, L.E., Bolanos, F. and Hamon, M. (1994) Differential effects of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) on various 5-HT receptor binding sites in the rat brain. *Neuropharmacol., 33:*423-431.

Henry, D.J. and White, F.J. (1992) Electrophysiological correlates of psychomotor stimulant-induced sensitization. *Annals NY Acad. Sci., 654:*88-100.

King, G.R., Joyner, C., Lee, T., Ellinwood, E.H. (1992) Intermittent and continuous cocaine administration: residual behavioral states during withdrawal. *Pharmacol. Biochem. Behav. 43:*243-248.

King, G.R., Kuhn, C., and Ellinwood, E.H. (1993) Dopamine efflux during withdrawal from continous or intermittent cocaine. *Psychopharmacol., 111*: 179-184.

Lyon, M. and Robbins, R. (1975) The action of central nervous system stimulant drugs; a general theory concerning amphetamine effects. In:Essman, W.B. and Valzelli, L. (eds) *Current developments in psychopharmacology, Vol 2.*, Spectrum Publications, New York, pp.79-163.

Martin-Iverson, M.T. and Iversen, S.D. (1989) Day and night locomotor activity effects during administration of (+)-amphetamine. *Pharmacol. Biochem. Behav., 34:*

465-471.

Martin-Iverson, M.T., Iversen, S.D. and Stahl, S.M. (1988a) Long-term motor stimulant effects of (+)-4-propyl-9-hydroxynaphthoxazine (PHNO), a dopamine D2 receptor agonist: interactions with a dopamine D1 receptor antagonist and agonist. *Eur. J. Pharmacol., 149:*25-31.

Martin-Iverson, M.T., Stahl, S.M. and Iversen, S.D. (1988b) Chronic administration of a selective dopamine D2 agonist: factors determining behavioural tolerance and sensitization. *Psychopharmacol., 95*: 534-539.

Meller, E., Goldstein, M. Friedhoff, A.J. and Schweitzer, J.W. (1986) N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ): A new tool to probe CNS receptor function. In M. Goldstein, K. Fuxe, I. Tabachnick (Eds), *Central D1 dopamine receptors*, Plenum Press, New York, pp. 121-136.

Nowak, G. (1990) Measurement of the receptor turnover using an irreversible inactivating method. Theoretical assumptions, limitations and practical realization. *Pol. J. Pharmacol. Pharm., 42:*433-440.

Parsons, L.H. and Justice, J.B. Jr. (1993) Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area and dorsal raphe nucleus following repeated cocaine admininstration. *J. Neurochem.,61:*1611-1619.

Reith, M.E.A., Benuck, M. and Lajtha, A. (1987) Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. *J. Pharmacol. Exper. Ther., 243:*281-287.

Robbins, T.W., and Sahakian, B.J. (1983) Behavioral effects of psychomotor stimulant drugs: Clinical and neuropsychological implications. In: Creese I (ed) *Stimulants: Neurochemical, behavioral, and clinical perspectives*. Raven Press, New York, pp 301-338.

Segal, D.S., and Schuckit, M.A. (1983) Animal models of stimulant induced psychosis. In: Creese I (ed) *Stimulants: neurochemical, behavioral and clinical perspectives*. Raven Press, New York, pp 131-168.

Sorg, B.A. and Kalivas, P.W. (1993) Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. *Neurosci, 53:*695-703.

Stewart, J. and Vezina, P. (1989) Microinjections of SCH 23390 into the ventral tegmental area and substantia nigra pars reticulata attenuate the development of sensitization to the locomotor activating effects of systemic amphetamine, *Brain Res., 495:* 401-406.

Vezina, P. (1993) Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: an *in vivo* microdialysis study in the rat. *Brain Res., 605:*332-337.

Vezina, P. and Stewart, J. (1990) Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. *Brain Res., 516:*99-106.

Ziriax, J.M., Snyder, J.R., Newland, M.C. and Weiss, B. (1993) Amphetamine modifies the microstructure of concurrent behaviour. *Exp. Clin. Psychopharmacol., 1:*121-132.

APPENDIX A. GENERAL METHODS

A.1. CHEMICALS USED

CHEMICALS	SUPPLIERS
acetic acid - glacial	BDH Chemicals (Toronto, ON)
acetic anhydride	Caledon Laboratories (Georgetown, ON)
acetonitrile, HPLC grade distilled in glass	BDH Chemicals
ascorbic acid	Fisher Scientific
bovine serum albumin	Sigma
butaclamol	RBI (Research Biochemicals International)
cocaine hydrochloride	BDH Chemicals
deoxycholate	Fisher Scientific
dopamine HCl	Sigma
ethanol (denatured)	Fisher Scientific
3,4-dihydroxyphenylacetic acid	Sigma
N-ethoxycarbonyl-2-ethoxy-1,2-dihyroquinoline (EEDQ)	Sigma
ethyl acetate	BDH chemicals
folin-phenol reagent	Sigma
homovanillic acid	Sigma
hydrochoric acid	Fisher Scientific
5-hydroxyindole-3-acetic acid	Sigma
^{3}H-ketanserin	Dupont, NEN Products
(-)-noradrenalin HCl	Sigma

nimodipine	Miles Pharmaceuticals Ltd
perchloric acid	Fisher Scientific
phosphoric acid	Fisher Scientific
polyethylene glycol 400	Fisher Scientific
potassium carbonate anhydrous	Fisher Scientific
potassium chloride	Fisher Scientific
propylene glycol	Fisher Scientific
raclopride	Astra Labs, Sweden
^{3}H-SCH23390	Dupont, NEN Products
R(+)-SCH 23390	RBI (Research Biochemicals International)
^{3}H-spiperone	Dupont, NEN Products
scintillation fluid (Ready Safe™)	Beckman Instruments Inc.
sodium bicarbonate	Fisher Scientific
sodium carbonate anhydrous	Fisher Scientific
sodium chloride	Fisher Scientific
sodium hydroxide	Fisher Scientific
sodium phosphate, dibasic, anhydrous	Fisher Scientific
sodium potassium titrate	Allen & Hanbury's (Toronto, ON)
tris(hydroxymethyl)aminomethane (TRIS)	Fisher Scientific

A.2. INSTRUMENTATION AND APPARATUS

A.2.1. Filtration

A Brandel Cell harvester equipped with Whatman GF/C filters was used for the filtration steps in all receptor binding assays. This harvester allowed for the simultaneous filtration of 48 samples.

A.2.2. Centrifuges

A Beckman L755 vacuum refrigerated ultracentrifuge was used for centrifugation of binding samples.

A.2.3. Tissue Homogenizer

A combination of a TRI-R S63C variable speed laboratory motor with a Teflon™ glass pestle and a glass grinding tube was used for homogenizing all tissue samples for HPLC analysis. For the sake of consistency, a setting of 7 was used at all times. The Ultra turrax homogenizer was used as described previously, for binding homogenates.

A.2.4. Weighing Balances

A Mettler AE 160 electronic balance was used for weighing chemicals and biological samples.

A.2.5. Glass Cleaning

All glassware was rinsed with tap water and washed with biodegradable Sparkleen™ (Fisher Scientific) solution. Further washing was accomplished with a dishwasher (Miele Electronic 6715). For test tubes, an additional step was added: test tubes were sonicated (ultra-sonic cleaner, Mettler Electronics) in a solution of Decon 75 concentrate (BDH

Chemicals) before the dishwasher wash. After removal from the dishwasher, all glassware was air-dried in a convection oven Model 28, Precision Scientific Group.

APPENDIX B. BEHAVIOUR DURATIONS (TABLE 1) AND FREQUENCIES (TABLE 2)

Appendix Table 1. Mean durations (sec) for each behaviour sampled and analyzed in Chapter 4. n=6 per drug group.

Behaviour	Vehicle				Cocaine 2.5 mg/day				Cocaine 7.5 mg/day				Cocaine 22.5 mg/day			
	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14
Stand	243	532	1033	937	331	543	1055	930	463	473	1624	966	909	535	2171	854
Locomote	16	12	30	24	13	13	27	17	22	19	62	32	27	16	187	45
Head Movement	233	222	628	425	221	343	699	386	406	263	1446	535	669	589	2363	580
Sniff	143	232	626	426	144	304	643	453	391	341	1433	622	681	352	2347	677
Contact	98	191	418	383	88	217	424	598	241	288	1006	439	335	227	1436	572
Mouth Movement	97	138	281	248	102	118	324	181	117	95	215	219	205	109	261	179
Rear	21	29	87	80	24	82	78	100	33	53	169	121	104	82	254	160
Gnaw	20	12	19	5	2	0.18	1.5	1.8	0	1.8	0	2.9	5	1.2	1.8	3
Scrabble	0	3.7	1.5	0	0	2.4	0.2	5.2	0	8.7	9.1	507	0	0	23	0.7
Jump	0.4	1.3	5.3	3.7	0	1	1.5	1.5	0	1	6.3	2.5	0.5	0.6	8.4	3.4
Shake	4.3	3.3	13	3.2	5.1	5.1	11	3.2	4.2	3.7	16	5.1	7	3.3	11	3.2
Stretch	12.5	16	25	27	15	21	35	15	21	10	23	13	12	11	13	18
Yawn	205	6	21	12	10	12	28	11	13	7	17	9	10	9	15	14
Scratch	69	175	293	178	99	181	359	149	147	263	280	175	125	160	77	201
Limb Flick	1.8	2.4	5.3	4.7	1.5	3.3	1.9	4	3.2	2.5	9.2	7.7	2.5	2.3	9.2	6.4
Groom	91	277	844	305	146	304	359	299	179	349	358	342	239	204	243	353

Behaviour	Vehicle				Cocaine 2.5 mg/day				Cocaine 7.5 mg/day				Cocaine 22.5 mg/day			
	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14
Sit	120	294	384	341	138	316	359	271	190	346	450	345	265	192	374	435
Lie	3186	2724	2011	2121	3066	2567	2037	2205	2895	2659	1519	2073	2236	2700	878	2078
Body Adj.	79	106	91	93	109	99	128	83	113	87	91	84	95	74	60	78
Twitch	15	9.5	25	39	37	18	54	12	15	11	52	16	11	10	41	14
Sleep	1869	1658	626	686	1455	1392	251	542	1268	1487	297	645	954	1332	102	656

Appendix Table 2. Mean frequency counts for each behaviour sampled and analyzed in Chapter 4. n=6 per drug group.

Behaviour	Vehicle				Cocaine 2.5 mg/day				Cocaine 7.5 mg/day				Cocaine 22.5 mg/day			
	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14
Stand	38	47	130	87	35	55	112	59	51	57	236	110	84	50	540	126
Locomote	17	16	38	32	13	18	41	21	25	23	91	44	38	21	315	57
Head Movement	38	45	111	98	42	68	123	88	72	59	135	116	116	65	131	109
Sniff	15	20	55	39	17	23	66	27	23	26	79	43	38	23	86	39
Contact	22	29	75	53	16	38	84	52	44	46	175	87	54	37	318	74
Mouth Movement	26	26	62	45	30	31	74	35	34	26	54	45	51	24	62	45
Rear	4	6.5	16	12	4	12	13	11	6	12	36	21	7	9	65	25
Gnaw	0.8	1.5	2	2	.8	.5	1	.8	0	0.7	0.2	1.7	1.3	0.3	1.33	1.5
Scrabble	0.2	0.7	0.7	0	0	1.5	0.2	1.7	0	3.2	1.3	3	0	0	10.5	0.7
Jump	3.7	6.8	20	13	0.3	4.7	12	7.7	0.5	6.2	41	12	3.3	3.8	46	15
Shake	7.5	9	24	8	10	10	22	6.6	9	6.6	30	10	13	5.5	23	8
Stretch	8	8	16	15	7	12	20	9	8.6	6.5	13	9	7.5	6.2	10	12
Yawn	8	6.8	19	9	8	11	23	9	10	6	15	8	8	7	15	12
Scratch	9	14	29	17	11	16	34	13	13	20	34	21	13	13	11	17
Limb Flick	2.7	4.8	10	8	2.5	5.7	14	6.7	4.8	4.2	17	15	5.7	4.2	17	13
Groom	6.8	15	33	20	12	14	32	17	11	17	39	26	15	14	23	23

Behaviour	Vehicle				Cocaine 2.5 mg/day				Cocaine 7.5 mg/day				Cocaine 22.5 mg/day			
	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14	Day 1	Day 14	Night 1	Night 14
Sit	18	24	65	32	19	32	51	26	22	25	94	53	34	23	142	51
Lie	15	20	21	19	19	20	25	18	19	18	24	15	22	16	28	17
Body Adj.	62	77	103	108	83	86	139	91	90	72	120	102	77	62	98	88
Twitch	28	20	54	28	33	36	48	24	28	21	49	31	21	20	24	27
Sleep	14	12	8	5	13	10	2.8	3.8	12	11	3	5	7.7	9	0.5	5.2